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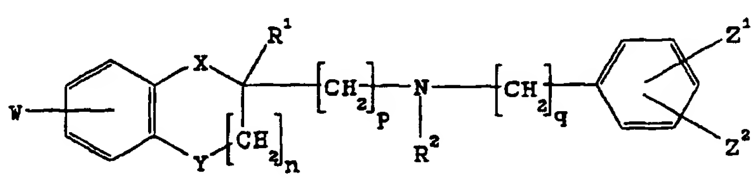
(54) Title: NOVEL AROMATIC AMINE DERIVATIVES



(I)

(57) Abstract

This invention relates to novel aromatic amine compounds having structure (I) where each W, Z^1 and Z^2 is independently H, C_1-C_6 alkyl, C_1-C_6 alkoxy, OH, F, Cl, Br, I, NO_2, CN, SO_2NHR^3, $NR^4{}_2$, $CONR^3{}_2$, COR^5; where each R^1 and R^2 is independently H, C_1-C_6 straight or branched chain alkyl or phenyl; where each X and Y is independently CH_2, NR^4, S, S=O, SO_2; where n is 0, 1 or 2; where each p and q is independently 1 or 2; where R^3 is H, C_1-C_6 straight or branched chain alkyl or phenyl; where R^4 is H, C_1-C_6 straight or branched chain alkyl or COR^3; and where R^5 is H, C_1-C_6 straight or branched chain alkyl or phenyl, C_1-C_6 straight or branched chain alkoxy or OH. In addition the invention includes using such compounds for the treatment of benign prostatic hyperplasia, lowering intraocular pressure and inhibiting cholesterol synthesis.

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Novel Aromatic Amine Derivatives

This application is a continuation-in-part of U.S. Serial No. 08/124,501, filed September 9, 1993 which was a continuation-in-part of U.S. Serial No. 07/975,867, filed November 13, 1992, the contents of which are hereby incorporated by reference.

Background of the Invention

Benign Prostatic Hyperplasia (BPH), also called Benign Prostatic Hypertrophy, is a progressive condition which is characterized by a nodular enlargement of prostatic tissue resulting in obstruction of the urethra. This results in increased frequency of urination, nocturia, a poor urine stream and hesitancy or delay in starting the urine flow. Chronic consequences of BPH can include hypertrophy of bladder smooth muscle, a decompensated bladder and an increased incidence of urinary tract infection. The specific biochemical, histological and pharmacological properties of the prostate adenoma leading to the bladder outlet obstruction are not yet known. However, the development of BPH is considered to be an inescapable phenomenon for the aging male population. BPH is observed in approximately 70% of males over the age of 70. Currently, in the United States, the method of choice for treating BPH is surgery (Lepor, H., Urol. Clinics North Amer., 17, 651 (1990)). Over 400,000 prostatectomies are performed annually (data from 1986). A medicinal alternative to surgery is clearly very desirable. The limitations of surgery for treating BPH include the morbidity rate of an operative procedure in elderly men, persistence or recurrence of obstructive and irritative symptoms, as well as the significant cost of surgery.

α-Adrenergic receptors are specific neuroreceptor proteins located in the peripheral and central nervous systems on tissues throughout the body. These receptors are important switches for controlling many physiological functions and, thus, represent important targets for drug development. In fact, many α-adrenergic drugs have been developed over the past 40 years. Examples include clonidine, phenoxybenzamine and prazosin (treatment of hypertension), naphazoline (nasal decongestant), and apraclonidine (treating glaucoma). α-Adrenergic drugs can be broken down into two distinct classes: agonists (clonidine and naphazoline are agonists), which mimic the receptor activation properties of the endogenous neurotransmitter norepinephrine, and antagonists (phenoxybenzamine and prazosin are antagonists), which act to block the effects of norepinephrine. Many of these drugs are effective but also produce unwanted side effects (for example, clonidine produces dry mouth and sedation in addition to its antihypertensive effects).

During the past 15 years a more precise understanding of α-adrenergic receptors and their drugs has evolved through increased scientific scrutiny. Prior to 1977, only one α-adrenergic receptor was known to exist. Between 1977 and 1988, it was accepted by the scientific community that at least two α-adrenergic receptors--α_1 and α_2--existed in the central and peripheral nervous systems. Since 1988, new techniques in molecular biology have led to the identification of at least six α-adrenergic receptors which exist throughout the central and peripheral nervous systems: α_{1A}, α_{1B}, α_{1C}, α_{2A}, α_{2B} and α_{2C} (Bylund, D.B., FASEB J., 6, 832 (1992)). It is not known precisely which physiological responses in the body are controlled by each of these receptors. In addition, many α-adrenergic drugs that were developed before 1992 are not selectiv for any particular α-adrenergic receptor. Many of these drugs produce untoward

side effects which may be attributed to their poor α-adrenergic receptor selectivity.

Since the mid 1970's, nonselective α-antagonists have been prescribed to treat BPH. In 1976, M. Caine, et al. (Brit. J. Urol., 48, 255 (1976)), reported that the nonselective α-antagonist phenoxybenzamine was useful in relieving the symptoms of BPH. This drug may produce its effects by interacting with α-receptors located on the prostate. However, this drug also produces significant side effects which severely limit its use in treating patients on a chronic basis. More recently, the α-adrenergic antagonists prazosin and terazosin have also been found to be useful for treating BPH. However, these drugs also produce untoward side effects. The most recently approved drug Proscar™ (Merck) prescribed for BPH is not an α-adrenergic antagonist, but rather acts by blocking 5-α-reductase. While Proscar is able to relieve symptoms, it is effective in only 30% of all patients, and requires a period of up to 6 months to show results.

From binding studies using cloned rat α_{1A}, hamster α_{1B}, and bovine α_{1C} receptors, and functional studies of antagonism <u>in vitro</u> using human prostate, Marshall, et al., concluded that the receptor mediating contraction of the human prostrate is of the α_{1C} subtype (Marshall, I., et al., Brit. J. Pharmacol., 107 (Proc. Suppl.) 327P, (1992)).

Furthermore, using cloned human receptors the binding characteristics of the known BPH drugs to various receptor subtypes have been determined, as described more fully hereinafter. Based upon such binding information and additional data, it has been observed that the side effects which occur with the drugs prazosin and terazosin may be due to their poor selectivity for specific α-adrenergic

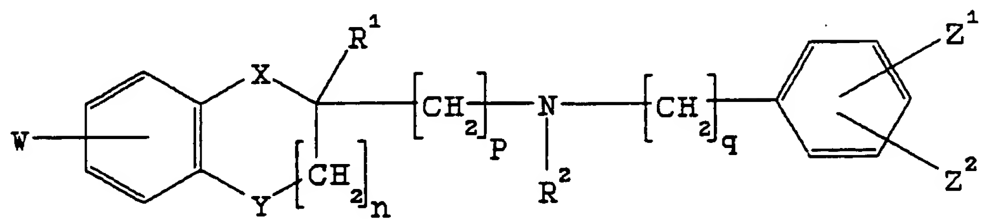
receptors. In contrast, indoramin is a drug which is slightly selective for the human α_{1C} receptor relative to the other human α-adrenergic receptors, but it also interacts at human histamine H_1 receptors. This compound produces untoward side effects which may be attributed to its activity at such H_1 receptors.

It would be desirable to provide methods and compounds which allow the treatment of BPH but which avoid the production of side effects observed for all currently used medications.

From the binding information described hereinafter, it has unexpectedly been discovered that compounds which are specific for an α_{1C} adrenergic receptor with a binding affinity greater than ten-fold higher than the binding affinity with which the compounds bind to an α_{1A} adrenergic receptor, a human α_{1B} adrenergic receptor, and a human histamine H_1 receptor, and bind to an α_2 adrenergic receptor with a binding affinity which is greater than ten-fold lower than the binding affinity with which the compounds bind to such α_{1C} adrenergic receptor are effective for the treatment of BPH. Building on this fundamental discovery the novel aromatic amines described herein were prepared.

Summary of the Invention

This invention relates to novel aromatic amine compounds having the structure:



where each W, Z^1 and Z^2 is independently H, C_1-C_6 alkyl, C_1-C_6 alkoxy, OH, F, Cl, Br, I, NO_2, CN, SO_2NHR^3, NR^4_2, $CONR^3_2$, COR^5; where each R^1 and R^2 is independently H, C_1-C_6 straight or branched chain alkyl or phenyl; where each X and Y is independently CH_2, NR^4, S, S=O, SO_2; where n is 0, 1 or 2; where each p and q is independently 1 or 2; where R^3 is H, C_1-C_6 straight or branched chain alkyl or phenyl; where R^4 is H, C_1-C_6 straight or branched chain alkyl or COR^3; and where R^5 is H, C_1-C_6 straight or branched chain alkyl or phenyl, C_1-C_6 straight or branched chain alkoxy or OH.

In addition the invention includes using such compounds for the treatment of benign prostatic hyperplasia, lowering intraocular pressure and inhibiting cholesterol synthesis.

Brief Description of the Drawings

A more complete understanding of the invention and many of its advantages will become apparent by reference to the detailed description which follows when considered in conjunction with the accompanying drawings, wherein:

Figure 1 illustrates compounds which are potent antagonists of the cloned human α_{1C} receptor.

Figures 2A, 2B and 2C illustrate the correlation of inhibition constants (pK_i) for a series of α_1 antagonists at the cloned human α_{1A}, α_{1B}, and α_{1C} receptors with efficiency of blocking contraction of human prostate tissue (pA_2).

Figure 3 illustrates a general synthetic scheme for the novel aromatic amine derivatives.

Detailed Description of the Inventi n

The present invention provides a method of treating benign prostatic hyperplasia in a subject which comprises administering to the subject a therapeutically effective amount of a compound which (a) binds to a human α_{1C} adrenergic receptor with a binding affinity greater than ten-fold higher than the binding affinity with which the compound binds to a human α_{1A} adrenergic receptor, a human α_{1B} adrenergic receptor, and a human histamine H_1 receptor, and (b) binds to a human α_2 adrenergic receptor with a binding affinity which is greater than ten-fold lower than the binding affinity with which the compound binds to such α_{1C} adrenergic receptor.

Desirably, the compound used to practice the method of the invention additionally binds to a calcium channel with a binding affinity which is greater than ten-fold lower than the binding affinity with which the compound binds to the α_{1C} adrenergic receptor.

Alternatively or incrementally, the compound used to practice the method of the invention also binds to a human dopamine D_2 receptor with a binding affinity which is greater than ten-fold lower than the binding affinity with which the compound binds to the α_{1C} adrenergic receptor.

Alternatively or incrementally, the compound used to practice the method of the invention additionally binds to a human histamine H_2 receptor with a binding affinity which is greater than ten-fold lower than the binding affinity with which the compound binds to the α_{1C} adrenergic receptor.

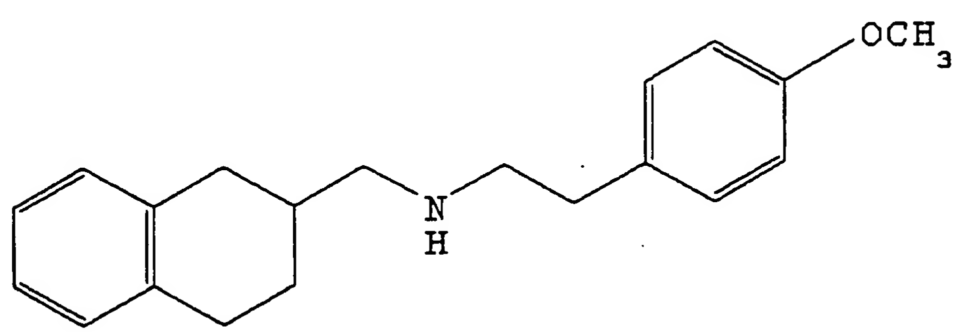
Alternatively or incrementally, the compound used to practice the method of the invention additionally binds to

any serotonin receptor with a binding affinity which is greater than ten-fold lower than the binding affinity with which the compound binds to the α_{1C} adrenergic receptor.

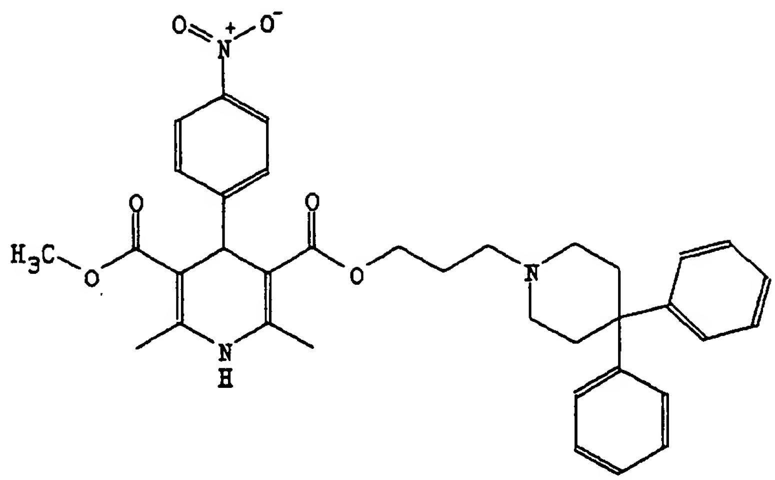
Alternatively or incrementally, the compound used to practice the method of the invention also binds to a human dopamine D_3 receptor with a binding affinity which is greater than ten-fold lower than the binding affinity with which the compound binds to the α_{1C} adrenergic receptor.

Alternatively or incrementally, the compound used to practice the method of the invention also binds to a human dopamine D_4 with a binding affinity which is greater than ten-fold lower than the binding affinity with which the compound binds to the α_{1C} adrenergic receptor.

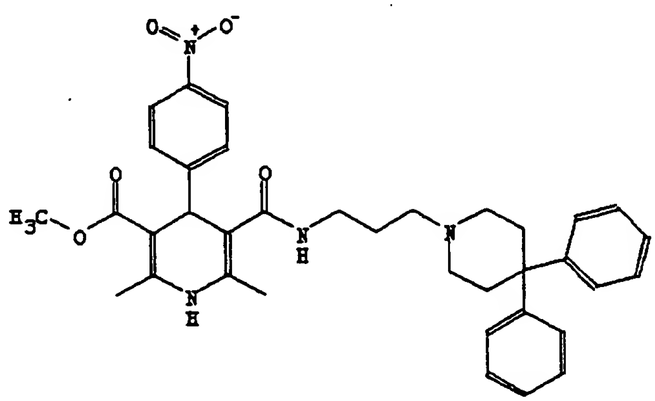
Alternatively or incrementally, the compound used to practice the method of the invention also binds to a human dopamine D_5 receptor with a binding affinity which is greater than ten-fold lower than the binding affinity with which the compound binds to the α_{1C} adrenergic receptor. A number of compounds have been identified or synthesized which are useful in the practice of the invention. For example, the compound having the structure:



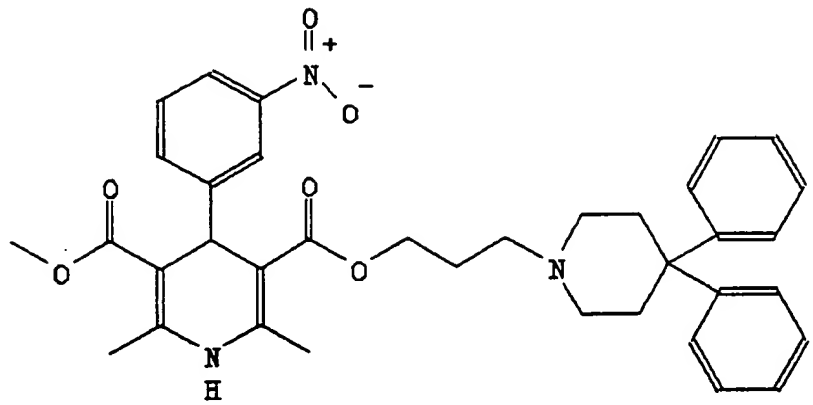
In another exampl , the compound has the structure:



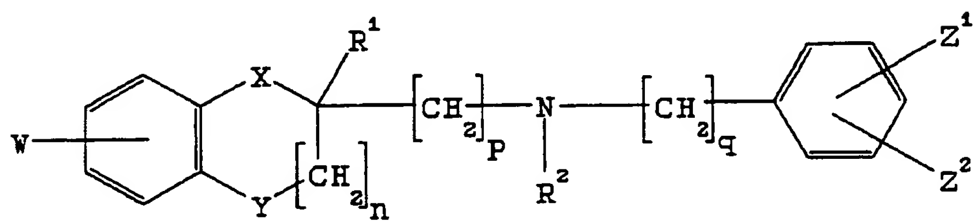
In still another example, the compound has the structure:



In an additional example, the compound has the structure:



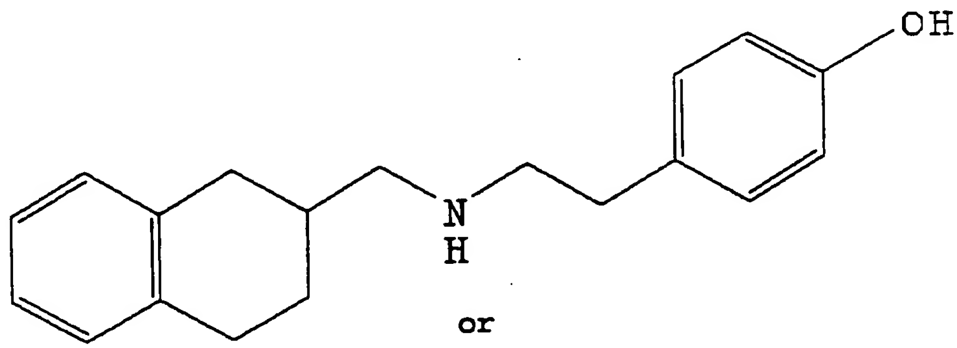
In addition the invention includes novel aromatic amine derivatives having the structure:



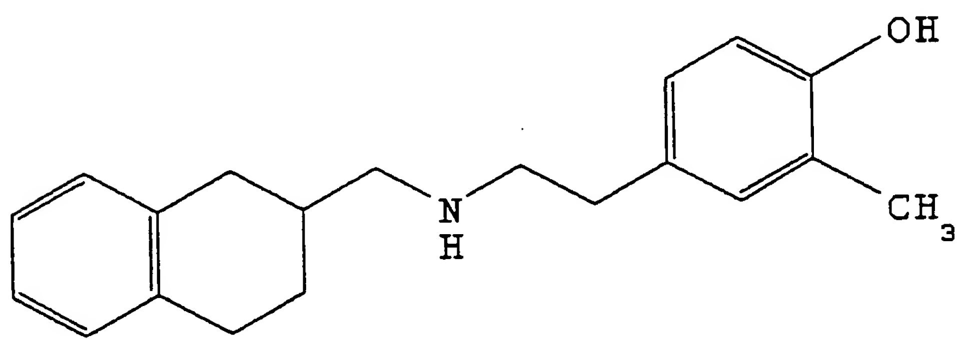
wherein each W, Z^1 and Z^2 is independently H, C_1-C_6 alkyl, C_1-C_6 alkoxy, OH, F, Cl, Br, I, NO_2, CN, SO_2NHR^3, NR^4_2, $CONR^3_2$, COR^5; wherein each R^1 and R^2 is independently H, C_1-C_6 straight or branched chain alkyl or phenyl; wherein each X and Y is independently CH_2, NR^4, S, S=O, SO_2; wherein n is 0, 1 or 2; each p and q is independently 1 or 2; wherein R^3 is H, C_1-C_6 straight or branched chain alkyl or phenyl; wherein R^4 is H, C_1-C_6 straight or branched chain alkyl or COR^3; and

wherein R^5 is H, C_1-C_6 straight or branched chain alkyl or phenyl, C_1-C_6 straight or branched chain alkoxy or OH.

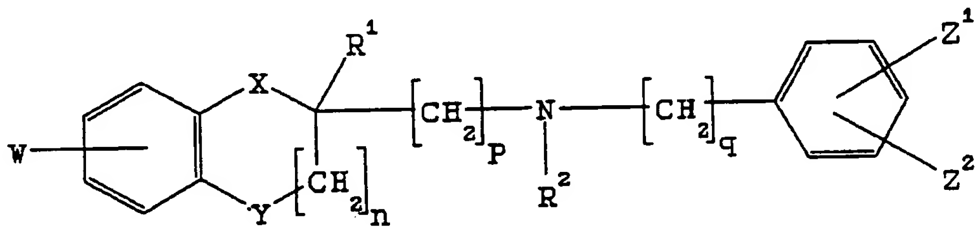
In one embodiment of the invention the compounds have the structure:



or



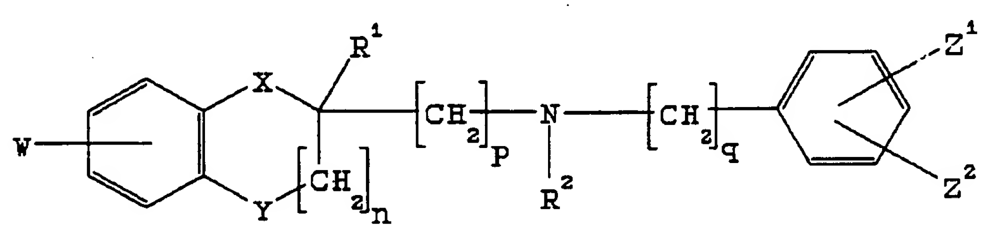
This invention also includes methods of treatment for benign prostatic hyperplasia, lowering intraocular pressure and for inhibiting cholesterol synthesis in a subject. Such methods comprise administering to a subject a compound having the structure:



wherein each W, Z^1 and Z^2 is independently H, C_1-C_6 alkyl, C_1-C_6 alkoxy, OH, F, Cl, Br, I, NO_2, CN, SO_2NHR^3, NR^4_2, $CONR^3_2$,

COR^5; wherein each R^1 and R^2 is independently H, C_1-C_6 straight or branched chain alkyl or phenyl; wherein each X and Y is independently CH_2, NR^4, C=O, S, S=O, SO_2; wherein n is 0, 1 or 2; wherein each p and q is independently 1 or 2; wherein R^3 is H, C_1-C_6 straight or branched chain alkyl or phenyl; wherein R^4 is H, C_1-C_6 straight or branched chain alkyl or COR^3; and wherein R^5 is H, C_1-C_6 straight or branched chain alkyl or phenyl, C_1-C_6 straight or branched chain alkoxy or OH.

The invention also provides for a method for treating benign prostatic hyperplasia in a subject which comprises administering to the subject an amount of an 5-alpha reductase inhibitor in combination with an amount of a compound having the structure:



wherein each W, Z^1 and Z^2 is independently H, C_1-C_6 alkyl, C_1-C_6 alkoxy, OH, F, Cl, Br, I, NO_2, CN, SO_2NHR^3, NR^4_2, $CONR^3_2$, COR^5; wherein each R^1 and R^2 is independently H, C_1-C_6 straight or branched chain alkyl or phenyl; wherein each X and Y is independently CH_2, NR^4, C=O, S, S=O, SO_2; wherein n is 0, 1 or 2; wherein each p and q is independently 1 or 2; wherein R^3 is H, C_1-C_6 straight or branched chain alkyl or phenyl; wherein R^4 is H, C_1-C_6 straight or branched chain alkyl or COR^3; and wherein R^5 is H, C_1-C_6 straight or branched chain alkyl or phenyl, C_1-C_6 straight or branched chain alkoxy or OH. For the combination method described above the 5-alpha reductase inhibitor may be finasteride.

Included within the scope of the method of treating BPH in accord with the invention are the use of both R and S enantiomers of the compounds described which possess stereogenic centers, as well as the use of pharmaceutically acceptable salts and complexes thereof.

The invention also provides a method of inhibiting contraction of prostate tissue which comprises contacting the prostate tissue with an effective contraction-inhibiting amount of a compound which (a) binds to a human α_{1C} adrenergic receptor with a binding affinity greater than ten-fold higher than the binding affinity with which the compound binds to a human α_{1A} adrenergic receptor, a human α_{1B} adrenergic receptor, and a human histamine H_1 receptor, and (b) binds to a human α_2 adrenergic receptor with a binding affinity which is greater than ten-fold lower than the binding affinity with which the compound binds to such α_{1C} adrenergic receptor.

The activity of compounds at the different human receptors was determined <u>in vitro</u> using cultured cell lines that selectively express the receptor of interest. These cell lines were prepared by transfecting the cloned cDNA or cloned genomic DNA or constructs containing both genomic DNA and cDNA encoding the human α-adrenergic, serotonin, histamine, and dopamine receptors as further described in detail in Example 26 hereinbelow.

In connection with this invention, a number of cloned human receptors discussed herein, either as plasmids or as stably transfected cell lines, have been made pursuant to, and in satisfaction of, the Budapest Treaty on the International Recognition of the Dep sit of Microorganisms for the Purpose of Patent Procedure, and are made with the American Type

Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852. Specifically, these deposits have been accorded ATCC Accession Numbers as follows:

Designation	ATCC Accession No.	Date
L-α_{1A}	CRL 11138	September 25, 1992
L-α_{1B}	CRL 11139	September 25, 1992
L-α_{1C}	CRL 11140	September 25, 1992
L-α_{2A}	CRL 11180	November 6, 1992
L-NGC-α_{2B}	CRL 10275	October 25, 1989
L-α_{2C}	CRL 11181	November 6, 1992
pcEXV-H_1	75346	November 6, 1992
pcEXV-H_2	75245	November 6, 1992
pcEXV-D_2	75344	November 6, 1992

The data shown in the accompanying Tables 1, 2 and 3 indicate that the α_{1C}-specific receptor antagonists which satisfy the criteria as defined herein have significant efficacy in the inhibition of contraction of human prostate tissue. This <u>in vitro</u> property is recognized in the art as correlating with efficacy in treating benign prostatic hyperplasia <u>in vivo</u>.

The present invention therefore provides a method of treating benign prostatic hyperplasia, lowering intraocular pressure or inhibiting cholesterol synthesis which comprises administering a quantity of any of the α_{1C} receptor antagonists defined as herein in a quantity effective against BPH, intraocular pressure or cholesterol synthesis. The drug may be administered by any conventional route of administration, including, but not limited to, intravenous, intramuscular, oral, subcutaneous, intratumoral, intradermal, and parenteral. The quantity effective against BPH is between 0.001 mg and 10.0 mg per kg of subject body weight and the quantity effective against intraocular pressure or cholesterol synthesis is between 0.001 mg and 10.0 mg per kg of subject body weight.

The method of treating BPH, lowering intraocular pressure or inhibiting cholesterol synthesis disclosed in the present invention may also be carried out using a pharmaceutical composition comprising any of the α_{1C} receptor antagonists as defined herein and a pharmaceutically acceptable carrier. The composition may contain between 0.05 mg and 500 mg of an α_{1C} receptor antagonist, and may be constituted into any form suitable for the mode of administration selected. Compositions suitable for oral administration include solid forms, such as pills, capsules, granules, tablets, and powders, and liquid forms, such as solutions, syrups, elixirs, and suspensions. Forms useful for parenteral administration include sterile solutions, emulsions, and suspensions.

The drug may otherwise be prepared as a sterile solid composition which may be dissolved or suspended at the time of administration using sterile water, saline, or other appropriate sterile injectable medium. Carriers are intended to include necessary and inert binders, suspending agents, lubricants, flavorants, sweeteners, preservatives, dyes, and coatings.

Optimal dosages to be administered may be readily determined by those skilled in the art, and will vary with the particular α_{1C} receptor antagonist in use, the strength of the preparation, the mode of administration, and the advancement of the disease condition. Additional factors depending on the particular patient being treated will result in a need to adjust dosages, including patient age, weight, diet, and time of administration.

The following Experimental Details are set forth to aid in an understanding of the invention, and are not intended, and

should not be construed, to limit in any way the invention set forth in the claims which follow thereafter.

Experimental Details.
Prazosin, 5-methylurapidil, and S-niguldipine were obtained from Research Biochemicals, Inc. A30360 (4-fluoro-4-(8-fluoro-1,3,4,5-tetrahydro-2H-pyrido[4,3-b]indol-2-yl)butyrophenone hydrochloride) was obtained from Aldrich Chemical Co. Other compounds were prepared according to the examples which follow.

Example 1

Synthesis of Terazosin Hydrochloride

N-(2-Furoyl)piperazine

This compound and its preparation have been described in Great Britain Patents 1,390,014 and 1,390,015.
Piperazine hexahydrate (194 g, 1 mole) was dissolved in 250 ml H_2O. The solution was acidified to pH 4.5 with 6 N HCl. Furoyl chloride (130.5 g, 1 mole, Aldrich) was added along with 10% NaOH solution at such a rate that the pH was maintained at 4.5. After 1 hour, the solution was made basic (pH = 8.5) with NaOH solution. The reaction mixture was continuously extracted with chloroform for 36 hours. The $CHCl_3$ extract was dried over $MgSO_4$, and filtered. Distillation gave 108.2 g product (60%), b.p. 132° - 138° C/0.6 mm Hg, mp 69° - 70 °C.

N-(Tetrahydro-2-furoyl)piperazine

The furoylpiperazine of Example 1 was converted to the hydrobromide salt (mp 173° - 175° C). This salt (39.0 g) in 250 ml methyl alcohol and 9.0 g Raney nickel was hydrogenated at 3 atm. After uptake of H_2 ceased, the catalyst was filtered, the solvent concentrated, and the residue crystallized from isopropyl alcohol to give 35.2 g of tetrahydrofuroylpiperazine HBr, mp 152° - 156 °C. This

was suspended in 20 ml H_2O. Then 10.5 g 50%, NaOH solution was added slowly followed by 2.0 g solid Na_2CO_3. This was extracted with 4 x 100 ml portions of warm $CHCl_3$. The $CHCl_3$ extractions were distilled to give 22.5 g tetrahydrofurolyl-piperazine, b.p. 120° - 125°C/0.2 mm Hg.

2[4-(Tetrahydro-2-furoyl)piperazinyl]-4-amino-6,7-dimethoxyquinazoline hydrochloride

To 7.00 g 2-chloro-4-amino-6,7-dimethoxyquinazoline (Lancaster Synthesis) in 50 ml methoxyethanol was added 10.8 g, tetrahydrofurolylpiperazine, and the mixture refluxed 3 hours. The clear solution was concentrated and an aqueous solution of potassium bicarbonate was added. The resultant solid that formed was filtered and washed with water. It was then added to methanol and the resulting suspension was acidified with a solution of hydrogen chloride in isopropyl alcohol. The resulting solution was concentrated and the residue crystallized from isopropyl alcohol giving 8.12 g. of product, mp 278° - 279°C.

Example 2

Preparation of Indoramin

4-Benzamido-1-[2-(3-indolyl)ethylpyridinium Bromide

A solution of 4-benzamidopyridine (1.98 g) and 3-(2-bromoethyl)indole (2.24 g) in EtOH (15 ml) was refluxed for 2 hours, and the crystallized product (3.13 g, mp 264 - 266°C) was collected by filtration from the hot reaction mixture. Recrystallization gave the hydrate.

3-[2-4-Benzamidopiperid-1-yl)ethyl]indole (Indoramin)

4-Benzamido-1-[2-(3-indolyl)ethyl]pyridiniumbromide (3.0g) in 91% EtOH (300 ml) containing Et_3N (0.8 g) was hydrogenated in the presence of freshly prepared W-7 Raney Ni catalyst (ca. 3 g) at 28.12 kg/cm^2 and 50° for 4 hours. After filtering off the catalyst, the filtrate was

evaporated and the residue was shaken with $CHCl_3$ and 2 N NaOH. The resulting insoluble material (1.61 g, mp 203 - 206°C) was collected and dried. Recrystallization from EtOH gave the product (1.34 g) as colorless needles.

Example 3

Preparation of 1-(3-benzoylpropyl)-4-benzamidopiperidine (Compound 9)

A mixture of 4-chlorobutyrophenone (447 mg, 2.45 mmol), 4-benzamidopiperidine (500 mg, 2.45 mmol) and K_2CO_3 (338 mg, 2.45 mmol) was heated up in boiling water bath for 1 hour. The reaction mixture was partitioned between water and $CHCl_3$. The organic layer was separated and dried over Na_2SO_4. After filtration and removal of solvent, the residue was purified by chromatography (SiO_2, MeOH:$CHCl_3$, 5:95). Recrystallization from AcOEt/hexane gave a white powder (78 mg, 8.2%). mp 143-144°C; ^{1}H NMR (CD_3OD, 400MHz) δ 1.65 (dq, J_1=3.16 Hz, J_2=11.9 Hz, 2H), 1.90-2.00 (m, 4H), 2.18 (t, J=11.9 Hz, 2H), 2.48 (m, 2H), 3.00-3.10 (m, 4H), 3.88 (m, 1H), 7.40-8.00 (m, 10H); Mass spectrum $(M+1)^+$ at m/z 351.

Example 4

Preparation of 1-[3-(4-chlorobenzoyl)propyl]-4-benzamidopiperidine (Compound 7)

A mixture of 3-(4-chlorobenzol)propyl bromide (640 mg, 2.45 mmol), 4-benzamidopiperidine (500 mg, 2.45 mmol) and K_2CO_3 (1.01 g, 7.34 mmol) in 50 ml of acetone was heated up to refluxing condition for 48 hours. The solid was removed by filtration. Concentration of filtrate in vacuo gave a yellowish solid, which was purified by chromatography (SiO_2, MeOH:$CHCl_3$, 5:95). 320 mg (33.9%) of white powder was obtained ^{1}H NMR ($CDCl_3$, 300 mHz) δ 1.46 (dq, J_1=1.0 Hz, J_2=8.4 Hz, 2H), 1.90-2.10 (m, 4H), 2.16 (m, 2H), 2.43 (t, J=6.9 Hz,

2H), 2.80-2.90 (m, 2H), 2.97 (t, J=6.9 Hz, 2H), 3.97 (m, 1H), 5.92 (d, J=7.8 Hz, 1H, N-H), 7.40-8.00 (m, 9H); Product was converted to HCl salt and recrystallized with MeOH/Et_2O, mp 243-244°C; Calcd for $C_{22}H_{25}ClN_2O_2 \cdot HCl \cdot H_2O$: C 60.15, H 6.37, N 6.37; Found: C 60.18, H 6.34, N 6.29.

Example 5

Preparation of SKF-104856

1-[(4-Chlorophenyl)thio]-2-propanone

Chloroacetone (32.3 g, 0.347 mol) was added to a mixture of 4-chlorothiophenol (50 g, 0.347 mmol) and sodium hydroxide (14 g, 0.347 mol) in water (400 ml) and the mixture was stirred at 25°C for 1 hour. The mixture was extracted with ethyl ether and the organic phase was washed with water, dried with magnesium sulfate and concentrated to give 69 g (99%) of 1-[(4-chlorophenyl)thio]-2-propanone.

5-Chloro-3-methylbenzo(b)thiophene

1-[(4-Cholorophenyl)thio]-2-propanone (50 g, 0.25 mol) was added to polyphosphoric acid (300 g) and the mixture was stirred as the temperature was gradually raised to 120°C as an exotherm started. The mixture was stirred at 130°C for 1 hour, diluted with water, extracted with ethyl ether and the organic phase was dried and concentrated. The residue was stirred in methanol (200 ml), filtered and the filtrate concentrated to give 17.5 g (40%) of 5-chloro-3-methylbenzo(b)thiophene: bp 120°C (0.6 mm Hg).

Ethyl 5-chloro-3-methylbenzo(b)thiophene-2-carboxylate

n-Butyllithium in hexane (2.6 M, 2.3 ml) was added to a solution of 5-chloro-3-methylbenzo(b)thiophene (1.0 g, 6 mmol) in ethyl ether (20 ml) stirred at 0°C under argon. The mixture was stirred for 30 minutes and transferred slowly under argon pressure to a stirred solution of ethyl chloroformate (0.63 g, 6 mmol) in ethyl ether (20 ml). The

mixture was stirred at 0°C for 30 minutes and at 25°C for 1.5 hours. The mixture was treated with water and the organic phase was dried, concentrated and triturated with hexane to give 1.0 g (67%) of ethyl 5-chloro-3-methyl-benzo(b)thiophene-2-carboxylate: mp 92.5 - 94 °C.

Ethyl3-bromomethyl-5-chlorobenzo(b)thiophene-2-carboxylate

A mixture of ethyl 5-chloro-3-methylbenzo(b)thiophene-2-carboxylate (9.0 g, 0.035 mol), N-bromosuccinimide (6.53 g, 0.037 mol) and benzoyl peroxide (130 mg) in carbon tetrachloride (150 ml) was refluxed and illuminated with a sunlamp for 2 hours. The resulting suspension was cooled, filtered and the filter cake was triturated with methanol to give 9.9 g, (85%) of the methanol-insoluble ethyl 3-bromomethyl-5-chlorobenzo(b)thiophene-2-carboxylate: mp 148-150°C.

Ethyl 5-Chloro-3-[N-(2,2-dimethoxyethyl)-N-methyl(amino-methyl)]benzol(b)thiophene-2-carboxylate

A mixture of ethyl 3-bromomethyl-5-chlorobenzo(b)thiophene-2-carboxylate (11 g, 0.033 mol), methylaminoacetaldehyde dimethyl acetal (4.76 g, 0.04 mol) and potassium carbonate (11.4 g, 0.8 mol) in dry acetone (200 ml) was stirred for 48 hours, filtered and the filtrate concentrated to give 11.8 g, (96%) of ethyl 5-chloro-3-(N-2,2-dimethoxyethyl)-N-methyl(aminomethyl)benzol(b)thiophene-2-carboxylate.

Ethyl 7-chloro-3,4-dihydro-4-methylthieno[4,3,2-ef]-[3]benzazepine-2-carboxylate

Ethyl 5-chloro-3-[N-(2,2-dimethoxyethyl)-N-methyl(aminomethyl)]benzo[b]thiophene-2-carboxylate (3.0 g, 8.1 mmol) was added in portions to trifluoromethanesulfonic acid (10 ml) stirred at 0°C under argon. The mixture was stirred at 25°C for 45 minutes and diluted with water. The mixture was basified with aqueous sodium hydroxide and

extracted with ethyl ether to give ethyl 7-chloro-3,4-dihydro-4-methylthieno-[4,3,2-ef][3]benzazepine-2-carboxylate.

Ethyl7-chloro-3,4,5,6-tetrahydro-4-methylthieno[4,3,2-ef]-[3]benzazepine-2-carboxylate

Diborane in tetrahydrofuran (1 M, 40 ml) was added to a solution of ethyl 7-chloro-3,4-dihydro-4-methylthieno[4,3,2-ef][3]benzazepine-2-carboxylate (2.8 g) in tetrahydrofuran (30 ml) stirred at 0°C. The mixture was refluxed for 3 hours and stirred at 25°C for 18 hours, cooled, treated with methanol (50 ml), refluxed for 18 hours and concentrated. The residue was triturated with ethyl ether-hexane (3:1) to give 1.6 g (84%) of ethyl 7-chloro-3,4,5,6-tetrahydro-4-methylthieno[4,3,2-ef][3]benzazepine-2-carboxylate: mp 138-140 °C. The free base was treated with hydrogen chloride to give ethyl 7-chloro-3,4,5,6-tetrahydro-4-methylthieno[4,3,2-ef][3]benzazepine-2-carboxylate hydrochloride: mp 240°C.

7-Chloro-3,4,5,6-tetrahydro-4-methylthieno[4,3,2-ef][3]benzazepine-2-methanol

A solution of ethyl 7-chloro-3,4,5,6-tetrahydro-4-methylthieno[4,3,2-ef][3]benzazepine-2-carboxylate (4.0 g, 12.9 mmol), in ethyl ether (48 ml) was treated with lithium aluminum hydride (0.53 g, 14 mmol). The mixture was stirred for 1.5 hours, cooled and treated carefully with water (2.0 ml), 10% sodium hydroxide (1.0 ml) and water (2.0 ml). The resulting mixture was filtered and the solvent evaporated to give 1.9 g (57%) of 7-chloro-3,4,5,6-tetrahydro-4-methylthieno[4,3,2-ef][3]benzazepine-2-methanol: mp 184-185°C.

7-Chloro-3,4,5,6-tetrahydro-4-methylthieno-4,3,2-ef][3]benzaz pine-2-carb xaldehyde

A solution of 7-chloro-3,4,5,6-tetrahydro-4-methylthieno[4,3,2-ef][3]benzazepine-2-methanol (1.6 g, 6

mmol) in dichloromethane (150 ml) was stirred under argon with activated manganese dioxide (8.3 g) for 2 hours. The mixture was filtered through Celite™ and the filtrate was dried with magnesium sulfate and concentrated to give a 63% yield of 7-chloro-3,4,5,6-tetrahydro-4-methylthieno[4,3,2-ef[[3]benzazepine-2-carboxaldehyde.

7-Chloro-2-ethenyl-3,4,5,6-tetrahdyro-4-methylthieno[4,3,2-ef][3]benzazepine (SKF-104856)

Sodium hydride (60 % dispersion in mineral oil, 3.8 mmol) was added to a stirred solution of methyltriphenylphosphonium bromide (1.35 g, 3.8 mmol) in dry tetrahydrofuran (30 ml) and stirred for 15 minutes. The mixture was treated with a solution of 7-chloro-3,4,5,6-tetrahydro-4-methylthieno[4,3,2-ef][3]-benzazepine-2-carboxaldehyde, prepared as in Example 3, (0.5 g, 1.9 mmol) in dimethylformamide (4 ml), stirred at 25°C for 16 hours, quenched with ice and extracted with ethyl acetate. The organic phase was washed, dried and concentrated and the residue was chromatographed on silica gel eluted with a gradient of methylene chloride to methanol-methylene chloride (3.5:96.5). The product was treated with hydrogen chloride to give 0.2 g (35%) of 7-chloro-2-ethenyl-3,4,5,6-tetrahydro-4-methylthieno[4,3,2-ef][3]benzazepine hydrochloride: mp 234-236°C.

Example 6

4,4-Diphenylpiperidine hydrochloride

A mixture of 4-piperidone monohydrate hydrochloride (15.0 g, 97.6 mmol, 1.00 equiv, Aldrich) and $AlCl_3$ (130 g, 976 mmol, 10.0 equiv) in anhydrous benzene (600 mL) was stirred at reflux for 4 hours. Ice (300 g) and water (50 mL) were added, th mixture was filtered, and the solid was washed with toluene and dried to afford 19.2 g (72%) of off-white solid, which was pure by ^1H NMR. Recrystallization from

ethanol gave the analytically pure sample: mp 300-301 °C; ^1H NMR (300 MHz, CD_3OD) δ 2.65 (m, 4 H), 3.18 (m, 4 H), 7.18 (m, 2 H), 7.30 (m, 8 H); Anal. Calcd. for $C_{17}H_{19}N{\cdot}HCl$: C, 74.57; H. 7.36; N, 5.12. Found: C, 74.32; H, 7.34; N, 5.02. The free base was generated by addition of the above salt to dilute aqueous sodium hydroxide and extraction with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and concentrated to give a light brown solid: IR (neat) 2942.8, 1494.5, 1445.9 cm^{-1}; CIMS (NH_3) m/e 238 $(M + 1)^+$.

3-(4,4-Diphenylpiperidin-1-yl)propionitrile

To a suspension of 4,4-diphenylpiperidine hydrochloride (195 mg, 0.712 mmol, 1.0 equiv) in ETOH (1.5 mL) was added triethylamine (0.25 mL, 1.83 mmol, 2.6 equiv) followed by acrylonitrile (0.13 mL, 2.01 mmol, 2.8 equiv). The resulting solution was stirred at room temperature under argon for 15 minutes and then concentrated. Water was added, and the mixture was extracted three times with EtOAc. The combined organic extracts were dried over $MgSO_4$ and concentrated to give 170 mg (87%) of tan solid, which was used for the next reaction without purification. mp 95-96°C; ^1H NMR (300 MHz, $CDCl_3$) δ 2.37 (m, 2H), 2,46 (m, 4H), 2.52 (m, 6H), 7.12 (m, 2H), 7.23 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.65, 36.71. 45.08, 50.78, 54.13, 119.70, 126.48, 127.78, 129.11, 147.87; IR (neat) 2944.4, 2821.0, 1495.5, 1445.9 cm^{-1}.

1-(3-Aminopropyl)-4,4-diphenylpiperidine

To a stirred solution of 3-(4,4-diphenylpiperidine-1-yl)propionitrile (2.00 g, 6.89 mmol, 1.0 equiv) in anhydrous THF (20 mL) under argon was added a solution of BH_3 in THF (1.0 M, 24.1 mL, 24 mmol, 3.5 equiv) at room temperature. The mixture was refluxed for 4.5 hours and then cooled to room temperature. Aqueous HCl (6 N, 50 mL) was added and stirring was continued for 1 hour. The mixture was basified

to pH 9 by addition of 6 N aq. NaOH, extracted 3 times with CH_2Cl_2, dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography (SiO_2, EtOAc-MeOH, 9:1, followed by EtOAc-MeOH-isopropylamine (60:10:1), followed by EtOAc-MeOH-isopropylamine (40:10:2) to give 1.35 g (66%) of tan solid: mp 98-99°C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.64 (tt, J=7.7 Hz, 2H), 2.33 (br t, J=7.2 Hz, 2H), 2.50 (m, 8H), 2.76 (br t, J=6.5 Hz, 2H), 3.06 (br s, 2H), 7.13 (m, 2H), 7.26 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.79, 36.80, 41.41, 45.24, 51.25, 57.41, 126.30, 127.77, 128.97, 148.11; IR (neat) 3361.5 cm^{-1}; CIMS (NH_3) m/e 295 $(M + 1)^+$.

Acetoacetic acid N-[3-(4,4-diphenylpiperidin-1-yl)propyl]amide

Diketene (0.44 mL, 5.68 mmol, 1.3 equiv, Aldrich) was added at room temperature to a stirred solution of 1-(3-aminopropyl)-4-,4-diphenylpiperidine (1.288 g, 4.37 mmol, 1.0 equiv) in anhydrous toluene (15 mL) under argon, and stirring was continued for 48 hours. The mixture was concentrated to give 1.294 g (78%) of white solid, which was used for the next reaction without purification: ^1H NMR (300 MHz, $CDCl_3$) δ 1.70 (tt, J=6.4, 6.4 Hz, 2H), 2.23 (s, 3H), 2.44 (br t, J=6.5 Hz), 2.49-2.67 (m, 8H), 3.32 (br t, J=5.8 Hz), 3.36 (s, 2H), 7.16 (m, 2H), 7.27 (m, 8H).

2,6-Dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid N-[3-(4,4-diphenylpiperidine-1-yl)propyl]amide methyl ester

A solution of acetoacetic acid N-[3-(4,4-diphenylpiperidin-1-yl)propyl]amide (365 mg, 0.964 mmol, 1.0 equiv), methyl 3-aminocrotonate (138 mg, 1.20 mmol, 1.2 equiv, Aldrich), and 4-nitrobenzaldehyde (181 mg, 1.20 mmol, 1.2 equiv, Aldrich) in isopropanol was refluxed under argon for 60 hours. The mixture was cooled to room temperature and concentrated, and the residu was diluted with CH_2Cl_2, washed with water, dried

over $MgSO_4$, and concentrated. The residue was purified by flash chromatography (SiO_2, EtOAc, followed by EtOAc-MeOH, 19:1 and 9:1) to give 147.8 mg (25%) of yellow solid: ^1H NMR (300 MHz, $CDCl_3$) δ 1.55 (m, 2H), 2.14 (s, 3H), 2.15-2.50 (m, 10H), 2.32 (s, 3H), 3.20 (m, 1H), 3.37 (m, 1H), 3.54 (s, 3H), 5.00 (s, 3H), 5.48 (br s), 6.98 (br t, J=4.9 Hz, 1H), 7.14-7.30 (m, 10H), 7.39 (dm, J=8.7 Hz, 2H), 8.05 (dm, J=8.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.74, 20.64, 25.61, 36.77, 40.20, 42.26, 45.03, 51.16, 51.61, 58.08, 100.65, 109.71, 124.35, 126.46, 127.61, 128.84, 129.06, 135.52, 146.96, 147.10, 154.55, 168.22, 168.70; IR (neat) 1680, 1610, 1515, 1340 cm^{-1}; MS (FAB) m/e 609 $(M + H)^+$.

2,6-Dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid N-[3-(4,4-diphenylpiperidin-1-yl)-propyl]amide methyl ester hydrochloride hydrate (Compound 2)

To a solution of 2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid N-[3-(4,4-diphenylpiperidin-1-yl)propyl]amide methyl ester (147.8 mg, 0.243 mmol, 1.0 equiv) in EtOH (2 mL) was added a solution of HCl in ether (1.0 M, 0.24 mL, 0.24 mmol, 1.0 equiv). Addition of ethyl acetate (3 mL) followed by heating gave a clear solution. Slow cooling of this solution followed by filtration gave 91 mg of yellow crystalline solid: mp 182-183°C; Anal. Calcd. for $C_{36}H_{40}N_4O_5 \cdot HCl \cdot H_2O$: C, 65.20, H, 6.54; N, 8.45. Found: C, 65.30; H, 6.28; N, 8.15.

Example 7

3-(4,4-Diphenylpiperid-1-yl)-propanol

4,4-Diphenylpiperidine (40 g), 3-bromopropanol (24.7 g, Aldrich), powdered potassium carbonate (116.4 g) and approximately 1 g of potassium iodide (in 500 ml of a 1:1 mixture of dioxane and 1-butanol) were heated for about 48 hours under reflux and with vigorous stirring. After cooling, the mixture was filtered, and the filtrate was

concentrated. The oily residue was taken up in ethyl acetate, and the solution was filtered again. Concentrating the filtrate to dryness yielded the product in the form of a yellowish, oily residue which slowly solidified to a wax-like product (yield: 44.8 g). Hydrochloric acid in ether produced the hydrochloride (mp: 226° to 227° C), which was recrystallized from 2-propanol.

Acetoacetic acid 3-(4,4-diphenylpiperidin-1-yl)propyl ester

23.6 g of 3-(4,4-diphenylpiperid-1-yl)-propanol was dissolved in 100 ml of absolute toluene, and 16 ml of a 50% strength solution of diketene in acetone was added with stirring. After standing for several days at room temperature (monitored by thin layer chromatography), the mixture was concentrated, and the residue was dried under high vacuum. The pale yellow, viscous oil which remains was employed without further purification for the next stage.

2,6-Dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxy-ylic acid [3-(4,4-diphenylpiperidin-1-yl)propyl] ester methyl ester

A solution of methyl 3-aminocrotonate (265 mg, 2.3 mmol, 1.0 equiv), 4-nitrobenzaldehyde (348 mg, 2.3 mmol, 1.0 equiv), and acetoacetic acid 3-[4,4-diphenylpiperidin-1-yl)propyl] ester (872 mg, 2.3 mmol, 1.0 equiv) in isopropanol was refluxed under argon with stirring for 68 hours. Cooling and removal of solvent gave a residue, which was purified by flash chromatography (SiO_2, EtOAc-hexane, 1:1 and 1:2, followed by EtOAc) to afford 717 mg (51%) of yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.73 (m, 2H), 2.22 (m, 2H), 2.30-2.51 (m, 8H), 2.34 (s, 3H), 2.35 (s, 3H), 3.63 (s, 3H), 4.05 (dt, J=2.1, 7.9 Hz, 2H), 5.06 (s, 1H), 5.73 (br s, 1H), 7.14 (m, 2H), 7.27 (m, 8H), 7.42 (dm, J=8.8 Hz, 2H), 8.06 (dm, J=8.8 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.30, 19.65, 26.32, 36.11, 39.88, 44.60, 50.60, 51.12, 55.34, 62.66, 102.99,

107.55, 123.39, 125.67, 127.12, 128.33, 128.65, 144.80, 144.93, 146.36, 147.50, 154.78, 166.91, 167.43; IR (neat) 1698.0, 1684.7, 1517.5, 1345.7 cm^{-1}; CIMS (NH_3) 610 $(M + 1)^+$, 553, 338.

2,6-Dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid [3-(4,4-diphenylpiperidin-1-yl)propyl] ester methyl ester hydrochloride (Compound 8)

To a solution of 2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid [3-(4,4-diphenylpiperidine-1-yl)-propyl] ester methyl ester (710 mg, 1.16 mmol, 1.0 equiv) in EtOH (5 mL) was added a solution of HCl in ether (1.0 M, 1.5 mL, 1.5 mmol, 1.3 equiv). The solvents were removed and the residue was dissolved in CH_2Cl_2. This solution was added dropwise to 25 mL of ether to afford, after filtration, 500 mg of yellow crystalline solid: mp 152-153°C. Anal. Calcd. for $C_{36}H_{39}N_3O_6\cdot HCl$: C, 66.92; H, 6.24; N, 6.50. Found: C, 66.70; H, 5.99; N, 6.27.

Example 8

2-[(4-Methoxyphenethyl)aminomethyl]-1,2,3,4-tetrahydronaphthalene (#11)

A solution of 1,2,3,4-tetrahydro-2-naphthoic acid (2.50 g, 14.2 mmol) in 100 ml THF was treated with $LiAlH_4$ (681 mg, 17.04 mmol) and the reaction mixture was heated under refluxing condition for 5 hrs. The suspension was cooled to 0 °C and quenched by addition of solid $Na_2SO_4\bullet 10H_2O$. The mixture was stirred at room temperature for 4 hrs. The solid was removed by filtration. Concentration of filtrate in vacuo gave 2-hydroxymethyl-1,2,3,4-tetrahydronaphthalene as a yellowish oil (2.28 g, 98.9%).

A solution of 2-hydroxymethyl-1,2,3,4-tetrahydronaphthalene (2.28 g, 14.0 mmol) in 100 ml of CH_2Cl_2 was treated with PBr_3 (1.28 g, 4.73 mmol) at 0°C. The mixture was stirred at room temperature for 72 hrs then

poured onto 100 g of ice. The organic layer was isolated, washed with 10% K_2CO_4 aqueous solution, H_2O, sat'd brine then dried over Na_2SO_4. After filtration and removal of solvent, the residue was purified by chromatography (SiO_2, AcOEt: hexane, 1:10) to give 2-bromomethyl-1,2,3,4-tetrahydronaphthalene as a colorless oil (1.33g, 41.6%).

A solution of 2-bromomethyl-1,2,3,4-tetrahydronaphthalene (1.33 g, 5.91 mmol), 4-methoxyphenethylamine (1.79 g, 11.8 mmol) in 50 ml of EtOH was refluxed for 48 hrs. After removal of EtOH <u>in vacuo</u>, the residue was dissolved in 100 ml of $CHCl_3$, washed with 10% K_2CO_3, H_2O, sat'd brine then dried over Na_2SO_4. Filtration followed by evaporation of solvent gave a yellow oil, which was purified by chromatography (SiO_2, MeOH: $CHCl_3$, 5:95) to give the final product as a yellowish oil (1.03 g, 58.9%). The product was converted to HCl salt, crystallization with MeOH/Et_2O gave a white powder; mp 274-275 °C; Calcd. for $C_{20}H_{25}NO$•HCl: C 72.37, H 7.91, N 4.22: Found: C 72.40, H 7.76, N 4.13.

EXAMPLE 9

2-[(4-Hydroxyphenethyl)aminomethyl]-1,2,3,4-tetrahydronaphthalene (#12)

The solution of 2-[(4-methoxyphenethyl)aminomethyl]-1,2,3,4-tetrahydronaphthalene (200 mg, 0.677 mmol) in 15 ml of dried CH_2Cl_2 was treatment with BBr_3 (1.69 ml 1M solution) at 0 °C. After stirring for 30 min., it was quenched with 2 ml of MeOH followed by treated with 15 ml sat'd $NaHCO_3$ solution. The organic layer was separated and concentrated <u>in vacuo</u> to give a yellowish oil, which was purified by chromatography (SiO_2, MeOH: $CHCl_3$, 5:95) to give a colorless oil (77 mg, 40%). The product was converted to HCl salt, crystallization with MeOH/Et_2O gave a white powder; mp 221-222 °C; Calcd. for $C_{19}H_{23}NO$•HCl•1/4H_2O: C 70.78, H 7.67, N 4.34; Found: C 70.66, H 7.31, N 4.25.

EXAMPLE 10

2-[Phenethylaminomethyl]-1,2,3,4-tetrahydronaphthalene (#13)

The suspension of 1,2,3,4-tetrahydro-2-naphthoic acid (500 mg, 2.84 mmol) in 5 ml of toluene was treated with oxalyl chloride (720 mg, 5.675 mmol). The mixture was stirred at 50 °C for 5 hrs. All volatile materials were removed in vacuo and product 1,2,3,4-Tetrahydro-2-naphthoyl chloride was obtained as a colorless oil, which was used for next step reaction without further purification.

The solution of phenethylamine (344 mg, 2.84 mmol) in 20 ml of CH_2Cl_2 was first treated with the solution of 1,2,3,4-tetrahydro-2-naphthoyl chloride (2.83 mmol) in 2 ml of CH_2Cl_2 followed by treatment with 10 ml 10% K_2CO_3 solution. After stirred at r.t. overnight, the organic layer was separated and washed with 2N HCl, water, sat'd brine and dried over Na_2SO_4. Filtration and removal of solvent gave N-(2-phenylethyl) 1,2,3,4-tetrahydro-2-naphthoic amide as a white powder (793 mg, 100%); mp 86-87 °C; Calcd for $C_{19}H_{21}NO$: C 81.67, H 7.59, N 5.01; Found: C 82.00, H 7.27, N 4.77.

The solution of N-(2-phenylethyl) 1,2,3,4-tetrahydro-2-naphthoic amide (693 mg, 2.48 mmol) in 15 ml of THF was treated with $LiAlH_4$ (149 mg, 3.72 mmol). The mixture was refluxed for 4 hrs then carefully quenched by addition of solid $Na_2SO_4 \bullet 10H_2O$. After stirred at r.t. overnight, the solid was removed by filtration and concentration of filtrate in vacuo gave a colorless oil (604 mg, 91.7%). The product was converted to HCl salt, crystallization with $MeOH/Et_2O$ gave a white powder; mp 260-263 °C; Calcd for $C_{19}H_{23}N \bullet HCl$: C 75.58, H 8.03, N 4.64: Found: C 75.77, H, 8.24, N 4.45.

EXAMPLE 11

2-[(4-Chloroph nethyl)aminom thyl]-1,2,3,4-tetrahydronaphthalene (#14)

The title compound was prepared using the method illustrated in Example 10.

Yield: 829 mg (98%), colorless oil. Conversion of product to HCl salt gave a white powder. mp 255 °C; Calcd for $C_{19}H_{22}ClN{\bullet}HCl$: C 67.85, H 6.91, N 4.16; Found: C 67.93, H 6.79, N 4.10.

EXAMPLE 12

2-[(4-Bromophenethyl)aminomethyl]-1,2,3,4-tetrahydronaphthalene (#15)

The title compound was prepared using the method illustrated in Example 10.

Yield: 879 mg (87%), colorless oil. Conversion of product to HCl salt gave a white powder. mp 260 °C; Calcd for $C_{19}H_{22}BrN{\bullet}HCl$: C 59.92, H 6.10, N 3.68; Found: C 60.01, H 6.17, N 3.66.

EXAMPLE 13

2-[(4-Fluorophenethyl)aminomethyl]-1,2,3,4-tetrahydronaphthalene (#16)

The title compound was prepared using the method illustrated in Example 10.

Yield: 665 mg (100%), colorless oil. Conversion of product to HCl salt gave a white powder. mp 259 °C; Calcd for $C_{19}H_{22}FN{\bullet}HCl$: C 71.34, H 7.26, N 4.38; Found: C 70.97, H 7.59, N 4.27.

EXAMPLE 14

2-[(4-Nitrophenethyl)aminom thyl]-1,2,3,4-tetrahydronaphthalene (#17)

The title compound was prepared using the method similar to Example 10, BH_3•THF was used instead of $LiAlH_4$.

The product, a colorless oil was converted to HCl salt to give a white powder(1.042 g, 75% yield); mp 224 °C; Calcd for $C_{19}H_{22}N_2O_2$•HCl: C 65.79, H 6.68, N 8.08; Found: C 65.71, H 6.53, N 7.77.

EXAMPLE 15

2-[(3-Methoxyphenethyl)aminomethyl]-1,2,3,4-tetrahydronaphthalene (#18)

The title compound was prepared using the method illustrated in Example 10.

Yield: 844 mg (95%), yellowish oil. Conversion of product to HCl salt gave a white powder. mp 213 °C; Calcd for $C_{20}H_{25}NO$•HCl: C 72.37, H 7.91, N 4.22; Found: C 72.20, H 7.92, N 4.14.

EXAMPLE 16

2-[(3-Hydroxyphenethyl)aminomethyl]-1,2,3,4-tetrahydronaphthalene (#19)

The title compound was prepared using the method illustrated in Example 9.

Yield: 52 mg (27%), colorless oil. Conversion of product to HCl salt gave a white powder. mp 178 °C; Calcd for

$C_{19}H_{23}NO$•HCl: C 71.78, H 7.62, N 4.41; Found: C 71.56, H 7.52, N 4.58.

EXAMPLE 17

2-[(3-Methylphenethyl)aminomethyl]-1,2,3,4-tetrahydronaphthalene (#20)

The title compound was prepared using the method illustrated in Example 10.

Yield: 400 mg (100%), yellowish oil. Conversion of product to HCl salt gave a white powder. mp 234 °C; Calcd for $C_{20}H_{25}N$•HCl: C 76.03, H 8.31, N 4.43; Found: C 75.99, H 8.30, N 4.36.

EXAMPLE 18

2-[(3-Fluorophenethyl)aminomethyl]-1,2,3,4-tetrahydronaphthalene (#21)

The title compound was prepared using the method illustrated in Example 10.

Yield: 755 mg (89%), yellowish oil. Conversion of product to HCl salt gave a white powder. mp 245 °C; Calcd for $C_{19}H_{22}FN$•HCl: C 71.34, H 7.26, N 4.38; Found: C 71.08, H 7.43, N 4.32.

EXAMPLE 19

2-[(3,4-Dimethoxyphenethyl)aminomethyl]-1,2,3,4-tetrahydr naphthalene (#22)

The title compound was prepared using the method illustrated in Example 10.

Yield: 833 mg (89%), yellowish oil. Conversion of product to HCl salt gave a white powder. mp 220 °C; Calcd for $C_{21}H_{27}NO_2$•HCl: C 69.68, H 7.81, N 3.87; Found: C 69.55, H 7.82, N 3.80.

EXAMPLE 20

2-[(3-Methyl-4-methoxyphenethyl)aminomethyl]-1,2,3,4-tetrahydronaphthalene (#23)

The title compound was prepared using the method illustrated in Example 10.

Yield: 780 mg (98%), yellowish oil. Conversion of product to HCl salt gave a white powder. mp 270 °C; Calcd for $C_{21}H_{27}NO$•HCl: C 72.90, H 8.17, N 4.05; Found: C 72.84, H 8.37, N 4.02.

EXAMPLE 21

2-[(3-Methyl-4-Hydroxyphenethyl)aminomethyl]-1,2,3,4-tetrahydronaphthalene (#24)

The title compound was prepared using the method illustrated in Example 9.

Yield: 115 mg (60%), colorless oil. Conversion of product to HCl salt gave a white powder. mp 271 °C; Calcd for $C_{20}H_{25}NO$•HCl: C 72.37, H 7.91, N 4.22; Found: C 72.20, H 7.94, N 4.09.

EXAMPLE 22

N-M thyl-N-[2-(1,2,3,4-tetrahydr naphthalyl)m thyl]-phenethylamin (#25)

The title compound was prepared using the method illustrated in Example 10.

Yield: 715 mg (80%), yellowish oil. Conversion of product to HCl salt gave a white powder. mp 96 °C; Calcd for $C_{20}H_{25}N \bullet HCl$: C 76.03, H 8.31, N 4.43; Found: C 75.95, H 8.44, N 4.36.

Example 23

BE2254 (HEAT) was prepared and analyzed by the following published methods:

1. Hansen, Werner. **Ger. Offen. 2,018,097,** (1970).

2. Benthe HF, Goethert M, Tuchinda P. Noradrenalin antagonistische Wirkung verschiedener Phenylaethylamin und Phenoxyaethylamin-Derivate. **Arzneimittelforsch** 1972; 22:1468-74.

3. Goethert M, Nolte J, Weinheimer G. Preferential blockade of postsynaptic α-adrenoceptors by BE 2254. **Eur. J. Pharmacol.** 1981; 70:35-42.

Example 24

Protocol for the Determination of the Potency of α_1 Antagonists

The activity of compounds at the different human receptors was determined in vitro using cultured cell lines that selectively express the receptor of interest. These cell lines were prepared by transfecting the cloned cDNA or cloned genomic DNA or constructs containing both genomic DNA and cDNA encoding the human α-adrenergic

α_{1A} Human Adrenergic Receptor: The entire coding region of α1A (1719 bp), including 150 basepairs of 5′ untranslated sequence (5′ UT) and 300 bp of 3′ untranslated sequence (3′ UT), was cloned into the BamHI and ClaI sites of the polylinker-modified eukaryotic expression vector pCEXV-3, called EXJ.HR. The construct involved the ligation of partial overlapping human lymphocyte genomic and hippocampal cDNA clones: 5′ sequence were contained on a 1.2 kb SmaI-XhoI genomic fragment (the vector-derived BamHI site was used for subcloning instead of the internal insert-derived SmaI site) and 3′ sequences were contained on a 1.3 kb XhoI-ClaI cDNA fragment (the ClaI site was from the vector polylinker). Stable cell lines were obtained by cotransfection with the plasmid α1A/EXJ (expression vector containing the α1A receptor gene) and the plasmid pGCcos3neo (plasmid containing the aminoglycoside transferase gene) into LM(tk$^-$), CHO, and NIH3T3 cells, using calcium phosphate technique. The cells were grown, in a controlled environment (37°C., 5% CO_2), as monolayers in Dulbecco's modified Eagle's Medium (GIBCO, Grand Island, NY) containing 25mm glucose and supplemented with 10% bovine calf serum, 100 units/ml penicillin g, and 100 μg/ml streptomycin sulfate. Stable clones were then selected for resistance to the antibiotic G-418 (1 mg/ml), and membranes were harvested and assayed for their ability to bind [^{3}H]prazosin as described below (see "Radioligand Binding assays").

α_{1B} Human Adrenergic Receptor: The entire coding region of α1B (1563 bp), including 200 basepairs and 5′ untranslated sequence (5′ UT) and 600 bp of 3′ untranslated sequence (3′ UT), was cloned into the EcoRI site of pCEXV-3 eukaryotic expression vector. The construct involved ligating the full-length containing EcoRI brainstem cDNA fragment from λ ZapII into the expression vector. Stable cell lines were selected as described above.

α_{1C} Human Adrenergic Receptor: The entire coding region of α1C (1401 bp), including 400 basepairs of 5′ untranslated sequence (5′ UT) and 200 bp of 3′ untranslated sequence (3′ UT), was cloned into the KpnI site of the polylinker-modified pCEXV-3-derived eukaryotic expression vector, EXJ.RH. The construct involved ligating three partially overlapping fragments: a 5′ 0.6kb HincII genomic clone, a central 1.8 EcoRI hippocampal cDNA clone, and a 3′ 0.6Kb PstI genomic clone. The hippocampal cDNA fragment overlaps with the 5′ and 3′ genomic clones so that the HincII and PstI sites at the 5′ and 3′ ends of the cDNA clone, respectively, were utilized for ligation. This full-length clone was cloned into the KpnI site of the expression vector, using the 5′ and 3′ KpnI sites of the fragment, derived from vector (i.e., pBluescript) and 3′-untranslated sequences, respectively. Stable cell lines were selected as described above.

Radioligand Binding Assays: Transfected cells from culture flasks were scraped into 5ml of 5mM Tris-HCl, 5mM EDTA, pH 7.5, and lysed by sonication. The cell lysates were centrifuged at 1000 rpm for 5 min at 4°C, and the supernatant was centrifuged at 30,000 x g for 20 min at 4°C. The pellet was suspended in 50mM Tris-HCl, 1mM $MgCl_2$, and 0.1% ascorbic acid at pH 7.5. Binding of the α1 antagonist [^{3}H]prazosin (0.5 nM, specific activity 76.2 Ci/mmol) to membrane preparations of LM(tk-) cells was done in a final volume of 0.25 ml and incubated at 37°C for 20 min. Nonspecific binding was determined in the presence of 10 μM phentolamine. The reaction was stopped by filtration through GF/B filters using a cell harvester. Inhibition experiments, routinely consisting of 7 concentrations of the tested compounds, were analyzed using a non-linear regression curve-fitting computer program to obtain Ki values.

α_2 Human Adrenergic Receptors: To determine the potency of α_1 antagonists at the α_2 receptors, LM(tk-) cell lines stably transfected with the genes encoding the α_{2A}, α_{2B}, and α_{2C} receptors were used. The cell line expressing the α_{2A} receptor is designated L-α_{2A}, and was deposited on November 6, 1992 under ATCC Accession No. CRL 11180. The cell line expressing the α_{2B} receptor is designated L-NGC-α_{2B}, and was deposited on October 25, 1989 under ATCC Accession No. CRL10275. The cell line expressing the α_{2C} receptor is designated L-α_{2C}, and was deposited on November 6, 1992 under ATCC Accession No. CRL-11181. Cell lysates were prepared as described above (see Radioligand Binding Assays), and suspended in 25mM glycylglycine buffer (pH 7.6 at room temperature). Equilibrium competition binding assays were performed using [3H]rauwolscine (0.5nM), and nonspecific binding was determined by incubation with 10μM phentolamine. The bound radioligand was separated by filtration through GF/B filters using a cell harvester.

Human Histamine H_1 Receptor: The coding sequence of the human histamine H_1 receptor, homologous to the bovine H_1 receptor, was obtained from a human hippocampal cDNA library, and was cloned into the eukaryotic expression vector pCEXV-3. The plasmid DNA for the H_1 receptor is designated pcEXV-H1, and was deposited on November 6, 1992 under ATCC Accession No. 75346. This construct was transfected into COS-7 cells by the DEAE-dextran method. Cells were harvested after 72 hours and lysed by sonication in 5mM Tris-HCl, 5mM EDTA, pH 7.5. The cell lysates were centrifuged at 1000 rpm for 5 min at 4°C, and the supernatant was centrifuged at 30,000 x g for 20 min. at 4°C. The pellet was suspended in 37.8 mM $NaHPO_4$, 12.2 mM KH_2PO_4, pH 7.5. The binding of the histamine H_1 antagonist [^{3}H]mepyramine (1nM, specific activity: 24.8 Ci/mM) was done in a final volume of 0.25 ml and incubated at room

temperature for 60 min. Nonspecific binding was determined in the presence of 10 μM mepyramine. The bound radioligand was separated by filtration through GF/B filters using a cell harvester.

Human Histamine H_2 Receptor: The coding sequence of the human H_2 receptor was obtained from a human placenta genomic library, and cloned into the cloning site of PCEXV-3 eukaryotic expression vector. The plasmid DNA for the H_2 receptor is designated pcEXV-H2, and was deposited on November 6, 1992 under ATCC Accession No. 75245. This construct was transfected into COS-7 cells by the DEAE-dextran method. Cells were harvested after 72 hours and lysed by sonication in 5mM Tris-HCl, 5mM EDTA, pH 7.5. The cell lysates were centrifuged at 1000 rpm for 5 min at 4°C, and the supernatant was centrifuged at 30,000 x g for 20 min at 4 °C. The pellet was suspended in 37.8 mM $NaHPO_4$, 12.2 mM $K2PO_4$, pH 7.5. The binding of the histamine H_2 antagonist [^{3}H]tiotidine (5nM, specific activity: 70 Ci/mM) was done in a final volume of 0.25 ml and incubated at room temperature for 60 min. Nonspecific binding was determined in the presence of 10 μM histamine. The bound radioligand was separated by filtration through GF/B filters using a cell harvester.

Human Serotonin Receptors: $5HT_{1D\alpha}$, $5HT_{1D\beta}$, $5HT_{1E}$, $5HT_{1F}$ Receptors: The cell lysates of LM(tk-) clonal cell line stably transfected with the genes encoding each of these 5HT receptor-subtypes were prepared as described above. The cell line for the $5HT_{1D\alpha}$ receptor, designated as Ltk-8-30-84, was deposited on April 17, 1990, and accorded ATCC Accession No. CRL 10421. The cell for the $5HT_{1D\beta}$ receptor, designated as Ltk-11, was deposited on April 17, 1990, and accorded ATCC Accession No. CRL 10422. The cell line for the $5HT_{1E}$ receptor, designated $5HT_{1E}$-7, was deposited on November 6,

1991, and accorded ATCC Accession No. 75344. The cell line for the $5HT_{1F}$ receptor, designated L-5-HT_{1F}, was deposited on December 27, 1991, and accorded ATCC Accession No. 10957. These preparations were suspended in 50mM Tris-HCl buffer (pH 7.4 at 37°C) containing 10 mM $MgCl_2$, 0.2 mM EDTA, 10μM pargyline, and 0.1% ascorbate. The potency of α_1 antagonists was determined in competition binding assay by incubation for 30 minutes at 37°C in the presence of 5nM [3H]serotonin. Nonspecific binding was determined in the presence of 10μM serotonin. The bound radioligand was separated by filtration through GF/B filters using a cell harvester.

Human $5HT_2$ Receptor: The coding sequence of the human $5HT_2$ receptor was obtained from a human brain cortex cDNA library, and cloned into the cloning site of pCEXV-3 eukaryotic expression vector. This construct was transfected into COS-7 cells by the DEAE-dextran method. Cells were harvested after 72 hours and lysed by sonication in 5mM Tris-HCl, 5mM EDTA, pH 7.5. This cell line was deposited with the ATCC on October 31, 1989, designated as L-NGC-$5HT_2$, and was accorded ATCC Accession No. CRL 10287. The cell lysates were centrifuged at 1000 rpm for 5 minutes at 4°C, and the supernatant was centrifuged at 30,000 x g for 20 minutes at 4°C. The pellet was suspended in 50mM Tris-HCl buffer (pH 7.7 at room temperature) containing 10 mM $MgSO_4$, 0.5mM EDTA, and 0.1% ascorbate. The potency of α_1 antagonists at $5HT_2$ receptors was determined in equilibrium competition binding assays using [3H]ketanserin (1nM). Nonspecific binding was defined by the addition of 10μM mianserin. The bound radioligand was separated by filtration through GF/B filters using a cell harvester.

Human Dopamine D_2 Receptors: The potency of α_1 antagonists at the D2 receptor was determined using membrane

preparations from COS-7 cells transfected with the gene encoding the human D_2 receptor. The coding region for the human D2 receptor was obtained from a human striatum cDNA library, and cloned into the cloning site of PCDNA 1 eukariotic expression vector. The plasmid DNA for the D_2 receptor is designated pcEXV-D2, and was deposited on November 6, 1992 under ATCC Accession No. 75344. This construct was transfected into COS-7 cells by the DEAE-dextran method. Cells were harvested after 72 hours and lysed by sonication in 5mM Tris-HCl, 5mM EDTA, pH 7.5. The cell lysates were centrifuged at 1000 rpm for 5 minutes at 4°C, and the supernatant was centrifuged at 30,000 x g for 20 minutes at 4°C. The pellet was suspended in 50 mM Tris-HCl (pH 7.4) containing 1mM EDTA, 5mM KCl, 1.5mM $CaCl_2$, 4mM $MgCl_2$, and 0.1% ascorbic acid. The cell lysates were incubated with [3H]spiperone (2nM), using 10μM (+)Butaclamol to determine nonspecific binding.

Other Dopamine receptors are prepared by known methods (D_3: Sokoloff, P. et al., Nature, 347, 146 (1990), and deposited with the European Molecular Biological Laboratory (EMBL) Genbank as X53944; D_4: Van Tol, H.H.M., et al., Nature, 350, 610 (1991), and deposited with EMBL Genbank as X58497; D_5: Sunahara, R.K., et al., Nature, 350, 614 (1991), and deposited with EMBL Genbank as X58454-HU HD 5DR).

Determination of the Activity of α_1 Antagonists at Calcium Channels

The potency of α_1 antagonists at calcium channels was determined in competition binding assays of [3H]nitrendipine to membrane fragments of rat cardiac muscle, essentially as described by Glossman and Ferry (Methods in Enzymology 109:513-550, 1985). Briefly, the tissue was minced and homogenized in 50mM Tris-HCl (pH 7.4) containing 0.1mM phenylmethylsulfonyl fluoride. The homogenates were

centrifuged at 1000 g for 15 minutes, the resulting supernatant was centrifuged at 45,000 g for 15 minutes. The 45,000 g pellet was suspended in buffer and centrifuged a second time. Aliquots of membrane protein were incubated for 30 minutes at 37°C in the presence of [3H]nitrendipine (1nM), and nonspecific binding was determined in the presence of 10μM nifedipine. The bound radioligand was separated by filtration through GF/B filters using a cell harvester.

Example 25

Functional Properties of α_1 Antagonists in the Human Prostate

The efficacy of α_1 adrenergic antagonists for the treatment of benign prostatic hyperplasia (BPH) is related to their ability to elicit relaxation of prostate smooth muscle. An index of this efficacy can be obtained by determining the potency of α_1 antagonists to antagonize the contraction of human prostatic tissue induced by an α_1 agonist "in vitro". Furthermore, by comparing the potency of subtype selective α_1 antagonists in binding assays using human α_1 receptors with their potency to inhibit agonist-induced smooth muscle contraction, it is possible to determine which of the α_1 adrenergic receptor subtypes is involved in the contraction of prostate smooth muscle.

Methods: Prostatic adenomas were obtained at the time of surgery from patients with symptomatic BPH. These were cut into longitudinal strips of 15mm long and 2-4 mm wide, and suspended in 5ml organ baths containing Krebs buffer (pH 7.4). The baths were maintained at 37°C and continuously oxygenated with 5% CO_2 and 95% O_2. Isometric tension was measured with a Grass Instrument FT03 force transducer interfaced with a computer. Tissue strips were contracted with varying concentrations of phenylephrine after incubating for 20 minutes in the absence and presence of at

least three different concentrations of antagonist. Dose-response curves for phenylephrine were constructed, and the antagonist potency (pA_2) was estimated by the dose-ratio method. The concentration of some antagonists in the tissue bath was assessed by measuring the displacement of [3H]prazosin by aliquots of the bath medium, using membrane preparations of the cloned human α_{1C} receptor. This control was necessary to account for losses of antagonist due to adsorption to the tissue bath and/or metabolism during the time the antagonists were equilibrated with the prostate tissue.

Results:

Table 1 shows that the pA_2 values measured for a series of α_1 antagonists in human prostate tissue correlate closely ($r=0.76$) with the corresponding pK_i values measured in the α_{1C} receptor assays. In contrast, the human prostate pA_2 values correlate poorly with the pK_i values measured at the α_{1A} ($r=-0.06$) and α_{1B} ($r=-0.24$) adrenergic receptors. (See Figure 2.) Thus, antagonists which are more potent at blocking the α_{1C} adrenergic receptor are more effective at blocking the contraction of the human prostate than antagonists which are more potent at the α_{1A} or α_{1B} adrenergic receptors. In addition, antagonists which are selective for the α_{1C} receptor will have a better therapeutic ratio than nonselective α antagonists.

With compound #11, the low pA_2 observed in the prostate may be attributed to tissue absorption or metabolism.

Table 2 illustrates the cross reactivity of α_1 antagonists at other receptors such as α_{2A}, α_{2B}, α_{2C}, histamine H_1, H_2, serotonin 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, 5-HT_{1E}, 5-HT_{1F}, 5-HT_2, and dopamine D_2. Only compounds 11, 8 and 2 hav binding affinities

which are greater than ten-fold higher at α_{1C} receptors than the binding affinities at other receptors.

Table 3 illustrates the binding potency (pK_i) of the novel aromatic amine compounds 11 - 25 for the α_{1A}, α_{1B}, α_{1C} and the α_{2A}, α_{2B}, α_{2C} receptors.

Table 4 illustrates the alpha adrenergic 'pKi' fold selectivity hAlpha1C 'n' receptors summary.

Table 5 illustrates the alpha adrenergic 'Ki(nM)' fold selectivity hAlpha1C 'n' receptors summary.

<u>**Example 26**</u>

Protocol for the determination of alpha 1C antagonists in the In-vivo canine prostate model (Somess et al) J. Vol. (1989), 141, 1230.

To demonstrate the efficiency of the alpha 1C antagonists, adult male mongrel dogs more than one year of age are chosen for the model. After induction of general anesthesia using sodium pentobarbital (25 mg/kg i.v.), the animals are incubated and allowed to breathe spontaneously. An arterial catheter is inserted via the femoral artery to monitor blood pressure and an i.v. line is inserted into the leg for fluid and drug administration. A constant saline infusion is maintained at 40 to 50 ml/hr. Next, a seven cm. lower abdominal incision is made one cm lateral to the penis, the bladder, prostate and a short segment of urethra is identified and isolated without damage to the nerves or blood vessels. A cystotomy incision is made through which the pressure catheter is inserted and positioned in the prostatic urethra. The cystotomy is not closed, but the wound edges are sutured to stop bleeding. The tip of the catheter is positioned just distal to the prostate and is

secured in place with an O-silk tie around the urethra. A second holding suture at the bladder neck secures the catheter in place.

An esophageal pressure catheter (Amdorfer Med. Spec. Inc., Greendale, WI), is used to measure closing pressures along the esophagus is easily adapted to our study. Fluid, either water or saline, is pumped by a Harvard infusion pump at 0.1 ml/min through a Gould pressure transducer into the catheter. The fluid exits at a port in the catheter which is in the prostatic urethra. Occlusion of the port, by contraction of the prostate, blocks the flow and a pressure wave is created. This pressure is transmitted back through the catheter to the transducer which is attached to a Gould recorder. Squeezing the prostate gland causes an increase in urethral pressure which verifies the correct position of the urethra. A response curve is first generated for epinephrine alone, in doses ranging from one mg/kg to 50 mg/kg The absolute rise in urethral pressure is recorded for each dose and the next dose is given when the urethral pressure returned to baseline. Epinephrine dose response curves are generated in all 18 animals tested. Increasing doses of alpha 1C are then given, and the epinephrine dose response curve repeated in the presence of each dose of antagonist. No animal receives more than one antagonist; six animals are used to test each antagonist. To test for tachyphylaxis, six separate dogs are challenged with repeated epinephrine doses for six hours, the usual length of each experiment.

From the dose-effect data, the inhibition constant (K_i) and the medium-effect dose (ED_{30}) is calculated. K_i is an overall potency constant of each antagonist which is not affected by the agonist dose. It is calculated from the double reciprocal plot. The median-effect dose (ED_{30}) is

obtained from the median-effect plot. The ED_{30} value is a relative potency index of each antagonist that is influenced by the dose of agonist. For a competitive antagonist, the ED_{30} value increases as the dose of agonist is increased. Both the K_i and ED_{30} values given are calculated by using microcomputer software and an IBM-PC.

Thus the compounds, particularly compounds #11, #12 and #24 efficiency in vivo is demonstrated.

Table 1.

COMPARISON OF THE BINDING POTENCY (pK_i) OF ALPHA-1 ANTAGONISTS IN CLONED HUMAN RECEPTORS AND THEIR PROTENCY (pA_2) TO INHIBIT PROSTATE SMOOTH MUSCLE CONTRACTION

Compound		Human Alpha-1 Adrenergic (pK_i)			Human Prostate (pA)
		α1A	α1B	α1C	
1	Prazosin	9.48	9.26	9.23	9.08
2	Compound 2	5.98	6.57	8.87	8.94
3	A-30360	7.49	7.86	8.52	8.72
4	5-Methyl-Urapidil	7.79	6.77	8.35	8.38
5	Indoramin	6.74	7.39	8.35	7.86
6	SKF-104856	8.48	7.50	7.60	7.66
7	Compound 7	6.82	7.18	8.42	7.63
8	Compound 8	6.52	7.07	8.48	7.46
9	Compound 9	6.12	6.76	7.83	7.41
10	Terazosin	8.46	8.71	8.16	7.30
11	Compound 11	6.81	7.14	8.36	6.64

TABLE 2.

Cross Reactivity of α_1 Antagonists at Cloned Human Receptors

(pKi)

Compound	α_1 Adrenergic			α_2 Adrenergic			Histamine	
	α1A	α1B	α1C	α2a	α2b	α2c	H1	H2
Terazosin	8.46	8.71	8.16	6.26	7.51	6.64	4.00	5.04
Prazosin	9.48	9.26	9.23	6.76	7.64	7.65	4.00	5.19
5-Methylurapidil	7.79	6.77	8.35	6.63	7.38	6.88	5.16	4.47
Indoramin	6.74	7.39	8.35	4.94	5.72	5.22	7.37	5.63
Compound 11	6.81	7.14	8.36	6.86	6.90	6.92	5.74	7.45
A-30360	7.49	7.86	8.52	6.69	6.37	6.23	6.03	5.77
Compound 7	6.82	7.18	8.42	6.19	6.07	6.09	7.59	6.02
Compound 9	6.12	6.76	7.83	5.80	5.69	5.90	7.29	5.44
SKF-104856	8.48	7.50	7.60	7.30	8.49	7.60	5.59	5.84
S-Niguldipine	6.72	7.07	8.75	6.19	5.24	6.43	6.78	6.24
Compound 8	6.52	7.07	8.48	5.99	6.12	5.77	6.67	6.11
Compound 2	5.98	6.57	8.87	5.48	5.93	5.88	7.16	7.48

ND: Not Determined

TABLE 2. (continued)

Cross Reactivity of α_1 Antagonists at Cloned Human Receptors

(pKi)

Compound	Serotonin					Dopamine	Calcium Channel
	5HT1Dα	5HT1Dβ	5HT1E	5HT1F	5HT2	D2	
Terazosin	<6.0	<6.0	<5.0	<5.0	<5.0	<5.0	5.19
Prazosin	<5.0	<5.0	ND	ND	<6.0	<5.0	4.57
5-Methylurapidil	7.30	6.82	ND	ND	<6.0	<5.0	ND
Indoramin	<6.0	<6.0	<5.0	<5.0	<7.0	<8.0	4.53
Compound 11	<6.0	<6.0	<5.0	<5.0	<7.0	<6.0	5.18
A-30360	<6.0	<6.0	<5.0	<5.0	<8.0	<9.0	5.26
Compound 7	<6.0	<5.0	<5.0	<5.0	<6.0	<7.0	4.79
Compound 9	<6.0	<6.0	<5.0	<5.0	<7.0	<7.0	4.44
SKF-104856	<7.0	<7.0	<6.0	<7.0	<6.0	<7.0	4.68
S-Niguldipine	ND	ND	ND	ND	<7.0	<7.0	8.04
Compound 8	<6.0	<5.0	<5.0	<5.0	<7.0	<6.0	6.87
Compound 2	<7.0	<6.0	<5.0	<5.0	<6.0	<7.0	6.13

ND: Not Determined

TABLE 3-1

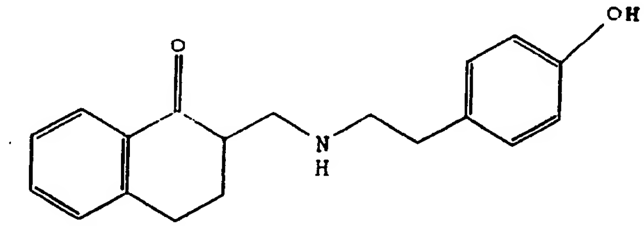
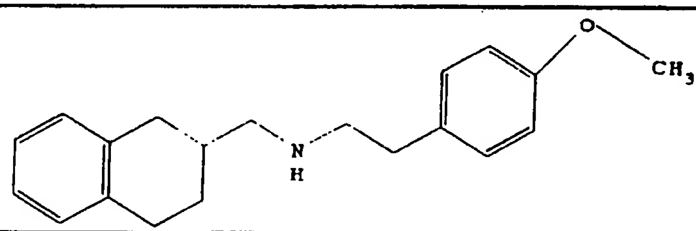
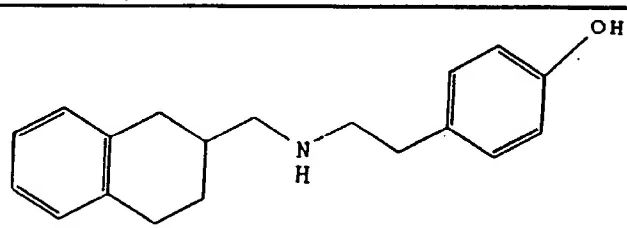
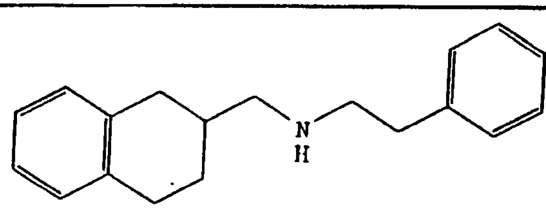
EXAMPLE	#		alpha-1			alpha-2		
			1a	1b	1c	2a	2b	2c
	BE	pKI	8.57	9.01	9	9.43	9.67	9.55
	2254*	SEM	0.09	0.15	0.08	0.22	0.17	0.35
		n	3	4	4	2	2	2
	11	pKI	6.81	7.14	8.36	6.86	6.9	6.92
		SEM	0.06	0.05	0.06	0.01	0.05	0.05
		n	3	3	3	2	2	2
	12	pKI	8.09	8.01	8.93	7.77	7.81	7.4
		SEM	0.05	0.03	0.05	0.19	0.12	0.08
		n	3	3	3	3	3	3
	13	pKI	6.98	7.19	7.74	6.81	7.25	6.64
		SEM	0.08	0.05	0.08	0.12	0.08	0.06
		n	3	3	3	3	3	3

TABLE 3-1 (continued)

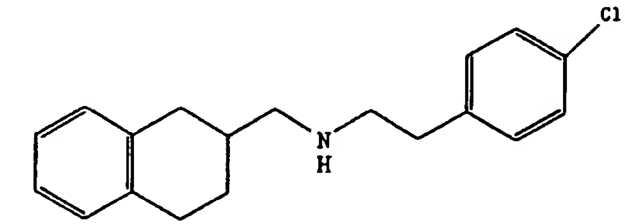
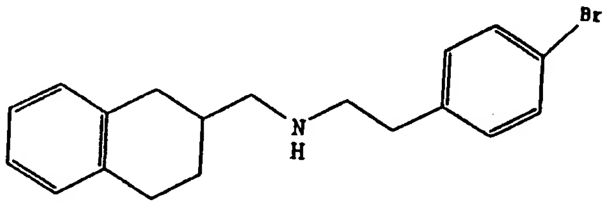
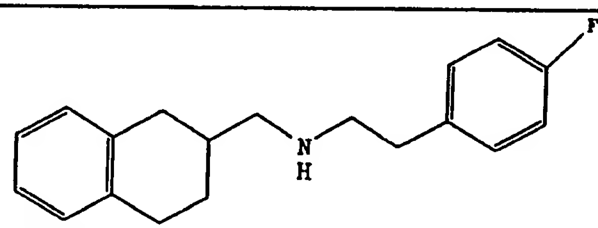
EXAMPLE	#		alpha-1			alpha-2		
			1a	1b	1c	2a	2b	2c
	14	pKI	7.15	7.54	8.26	6.55	6.8	6.48
		SEM	0.05	0.06	0.12	0.14	0.01	0.13
		n	3	3	3	3	3	3
	15	pKI	6.93	7.58	8.18	6.43	6.76	6.73
		SEM	0.14	0.12	0.12	0.01	0.06	0.04
		n	3	3	3	3	3	3
	16	pKI	7.09	7.4	7.9	6.74	7.15	6.83
		SEM	0.07	0.03	0.08	0.07	0.03	0.03
		n	3	4	4	3	3	3

TABLE 3-2

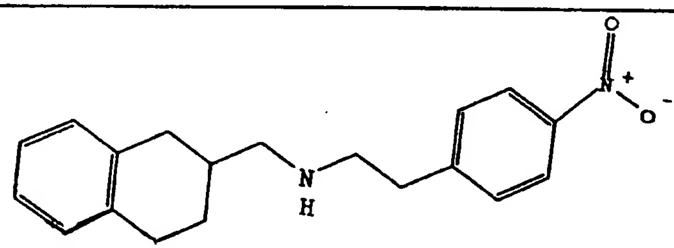
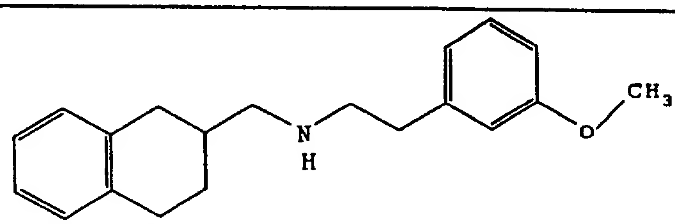
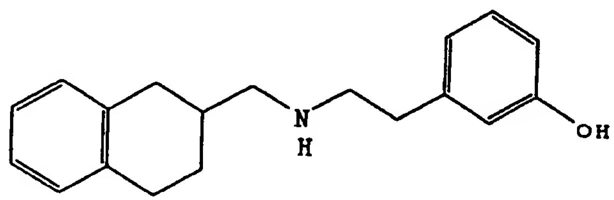
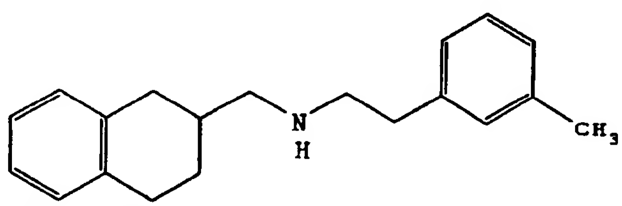
EXAMPLE	#		alpha-1			alpha-2		
			1a	1b	1c	2a	2b	2c
	17	pKI	6.87	7.3	7.89	6.82	6.98	6.74
		SEM	0.01	0.08	0.05	0.02	0.01	0.05
		n	3	3	3	3	3	3
	18	pKI	7	7.19	7.94	6.66	7.01	6.89
		SEM	0.16	0.05	0.04	0.1	0.02	0.07
		n	3	3	3	3	3	3
	19	pKI	7.59	7.5	8.33	7.16	7.34	7.35
		SEM	0.02	0.03	0.02	0.08	0.03	0.02
		n	3	4	4	3	3	3
	20	pKI	7.09	7.39	7.77	6.62	7.17	6.89
		SEM	0.06	0.03	0.04	0.03	0.02	0.04
		n	3	4	4	3	3	3

TABLE 3-2 (continued)

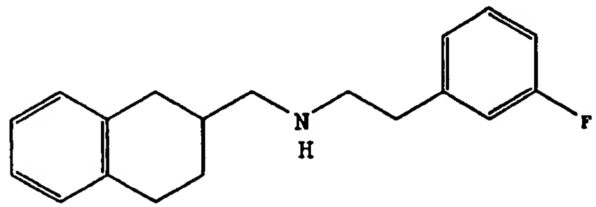
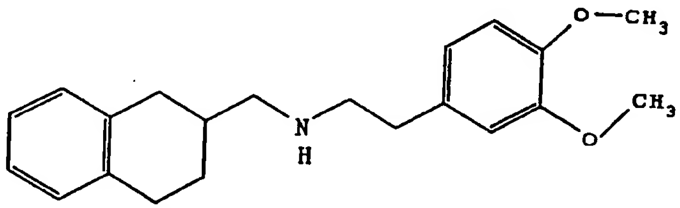
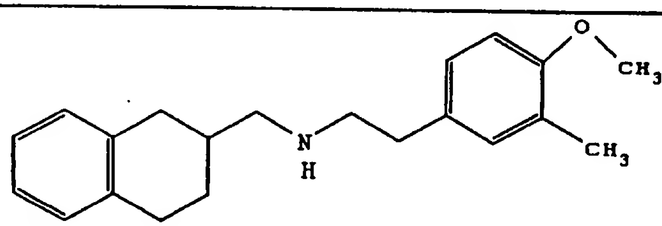
EXAMPLE	#		alpha-1			alpha-2		
			1a	1b	1c	2a	2b	2c
	21	pKI	6.83	7.17	7.7	6.72	7.16	6.78
		SEM	0.06	0.01	0.04	0.01	0.02	0.05
		n	3	4	4	3	3	3
	22	pKI	7.1	7.04	8.1	6.7	7.16	6.8
		SEM	0.01	0.24	0.06	0.08	0.05	0.03
		n	3	3	3	4	4	4
	23	pKI	7.33	7.41	7.94	6.55	6.94	6.57
		SEM	0.19	0.04	0.06	0.03	0.01	0.06
		n	3	4	4	3	3	3

TABLE 3-3

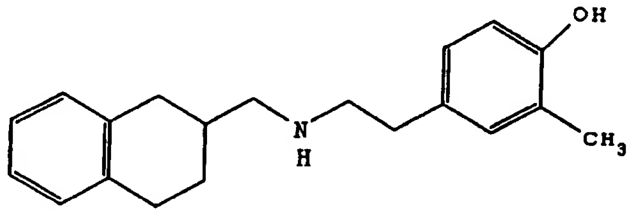
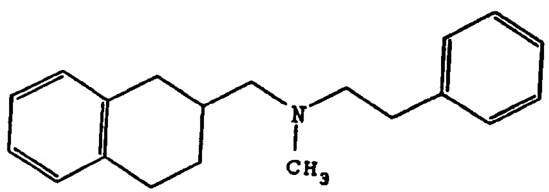
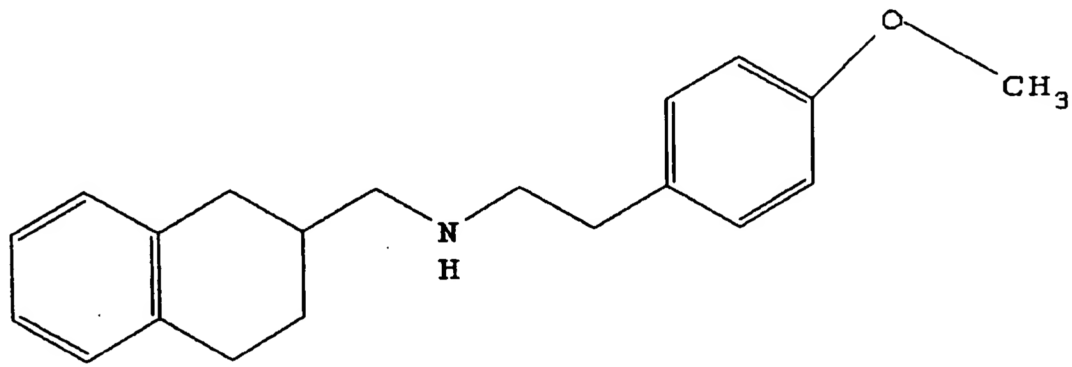
EXAMPLE	#		alpha-1			alpha-2		
			1a	1b	1c	2a	2b	2c
	24	pKI	8.12	8.08	8.85	7.38	7.63	7.64
		SEM	0.02	0.02	0.05	0.1	0.05	0.03
		n	3	3	3	3	3	3
	25	pKI	6.51	6.47	7.24	7.38	7.58	7.91
		SEM	0.01	0.05	0.05	0.06	0.08	0.22
		n	3	3	3	3	3	3

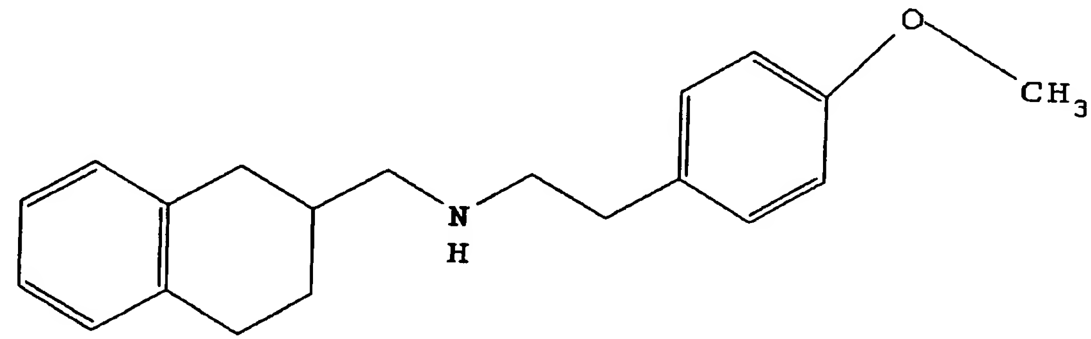
TABLE 4. Alpha adrenergic 'pKi' Fold selectivity hAlpha1c' n' RECEPTORS SUMMARY



hA1a 6.81 36 3	hA1b 7.14 17 3	hA1c 8.36 1 3	rA1a	rA1b	rA1c 1	dA1a	dA1b	dA1c 1	hH1 5.84 331 2	hH2 7.45 8 2	
hA2a 6.86 32 2	hA2b 6.91 29 2	hA2a 6.93 27 2	rA2a	rA2b	rA2c		Ca%1,A AghA1a AnthA1a	Ca%1,A Agha1b AnthA1b	Ca%1,A AghA1c AnthA1c		
hD1 0	hD2 0	hD3 0	hD5 0	h5HT1a 0	h5HT1Da 0	h5HT1Db 0	h5HT1E 0	h5HT1F 0	h5HT2 0	h5HT7 0	CaCh1 5.32 1096 1

TABLE 4. Alpha adrenergic 'pKi' Fold selectivity hAlphalc' n' RECEPTORS SUMMARY (continued)

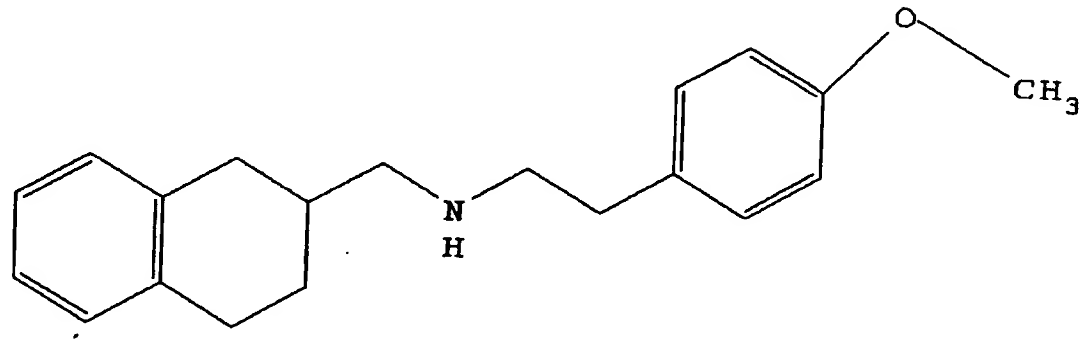
(+)



hA1a 6.60 19 3	hA1b 6.81 12 3	hA1c 7.87 1 3	rA1a	rA1b	rA1c 1	dA1a	dA1b	dA1c 1	hH1 0	hH2 0	
hA2a 6.70 15 3	hA2b 7.01 7 3	hA2a 7.03 7 3	rA2a	rA2b	rA2c		Ca%1,A AghA1a AnthA1a	Ca%1,A Agha1b AnthA1b	Ca%1,A AghA1c AnthA1c		
hD1 0	hD2 0	hD3 0	hD5 0	h5HT1a 0	h5HT1Da 0	h5HT1Db 0	h5HT1E 0	h5HT1F 0	h5HT2 0	h5HT7 0	CaCh1 5.69 153 1

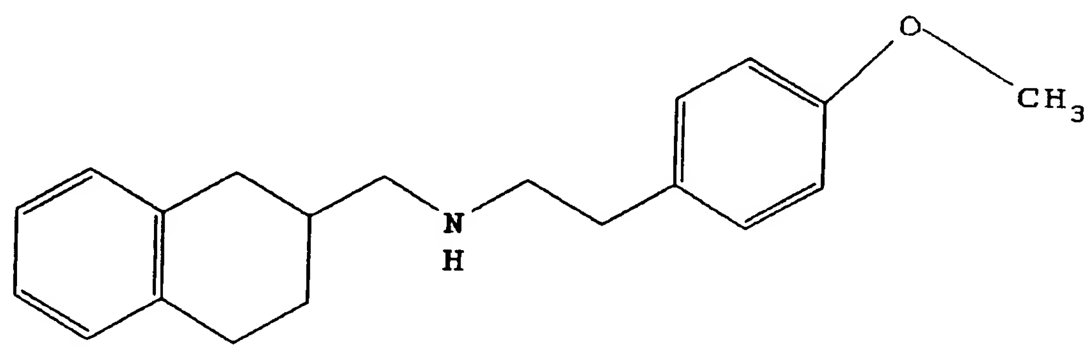
TABLE 4. Alpha adrenergic 'pKi' Fold selectivity hAlpha1c' n' RECEPTORS SUMMARY (continued)

(-)



hA1a 7.13 36 3	hA1b 7.68 10 3	hA1c 8.69 1 3	rA1a	rA1b	rA1c 1	dA1a	dA1b	dA1c 1	hH1 0	hH2 0	
hA2a 7.20 31 3	hA2b 7.30 25 3	hA2a 7.68 10 3	rA2a	rA2b	rA2c		Ca%1,A AghA1a AnthA1a	Ca%1,A Agha1b AnthA1b	Ca%1,A AghA1c AnthA1c		
hD1 0	hD2 0	hD3 0	hD5 0	h5HT1a 0	h5HT1Da 0	h5HT1Db 0	h5HT1E 0	h5HT1F 0	h5HT2 0	h5HT7 0	CaCh1 6.10 392 1

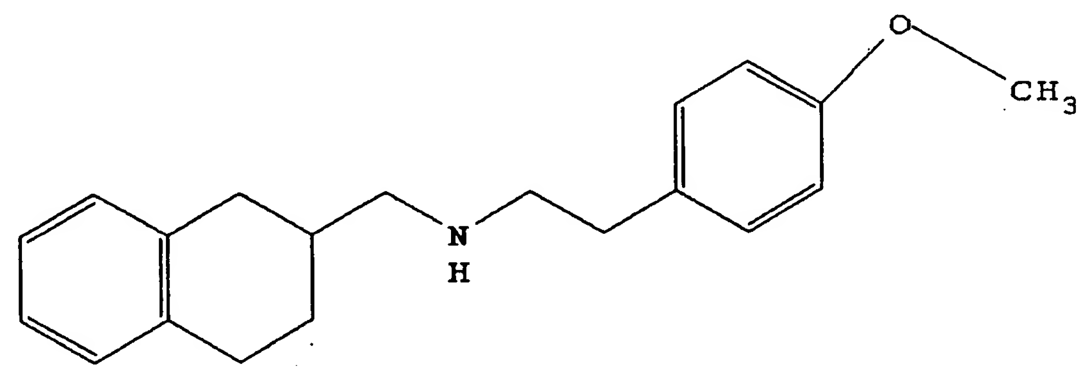
TABLE 5. Alpha adrenergic 'Ki (nM)' 'Fold selectivity hAlphalc' 'n' RECEPTORS SUMMARY



Ki Fold n	hA1a 156 36 3	hA1b 72 17 3	hA1c 4.4 1 3	rA1a	rA1b	rA1c 1	dA1a	dA1b	dA1c 1		
Ki Fold n	hA2a 138.0 32 2	hA2b 124.5 29 2	hA2c 118.9 27 2	hH1 1445 331 2	hH2 35 8 2	CaChl 4786 1096 1	%i.a. sem n	Ca%i.a. AghA1a AnthA1a	Ca%i.a. AghA1b AnthA1b	Ca%i.a. AghA1c AnthA1c	
Ki F ld n	hD1 0	hD2 0	hD3 0	hD5 0	h5HT1a 0	h5HT1Da 0	h5HT1Db 0	h5HT1E 0	h5HT1F 0	h5HT2 0	h5HT7 0

TABLE 5. Alpha adrenergic 'Ki (nM)' 'Fold selectivity hAlpha1c' 'n' RECEPTORS SUMMARY (continued)

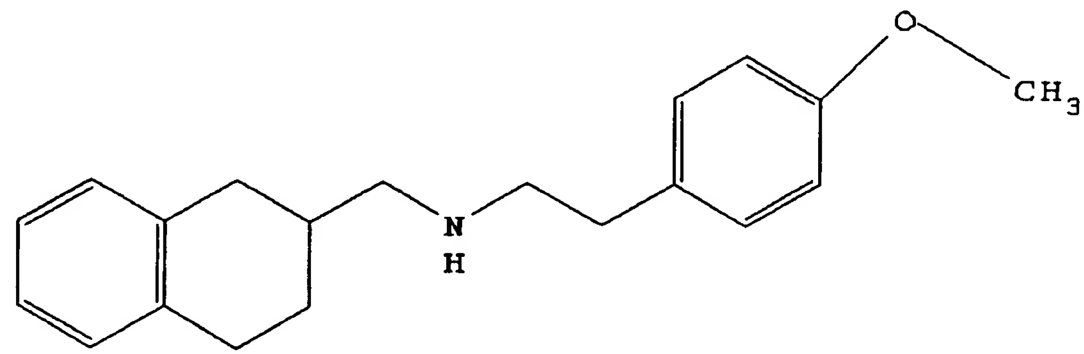
(+)



Ki Fold n	hA1a 249 19 3	hA1b 155 12 3	hA1c 13.4 1 3	rA1a	rA1b	rA1c 1	dA1a	dA1b	dA1c 1		
Ki Fold n	hA2a 201.1 15 3	hA2b 97.7 7 3	hA2c 93.3 7 3	hH1 0	hH2 0	CaCh1 2042 153 1	%i.a. sem n	Ca%i.a. AghA1a AnthA1a	Ca%i.a. Agha1b AnthA1b	Ca%i.a. AghA1c AnthA1c	
Ki Fold n	hD1 0	hD2 0	hD3 0	hD5 0	h5HT1a 0	h5HT1Da 0	h5HT1Db 0	h5HT1E 0	h5HT1F 0	h5HT2 0	h5HT7 0

TABLE 5. Alpha adrenergic 'Ki (nM)' 'Fold selectivity hAlpha1c' 'n' RECEPTORS SUMMARY (continued)

(-)



Ki Fold n	hA1a 74 36 3	hA1b 21 10 3	hA1c 2.0 1 3	rA1a	rA1b	rA1c 1	dA1a	dA1b	dA1c 1		
Ki Fold n	hA2a 63.1 31 3	hA2b 50.1 25 3	hA2c 21.1 10 3	hH1 0	hH2 0	CaCh1 794 392 1	%i.a. sem n	Ca%i.a. AghA1a AnthA1a	Ca%i.a. Agha1b AnthA1b	Ca%1,A AghA1c AnthA1c	
Ki Fold n	hD1 0	hD2 0	hD3 0	hD5 0	h5HT1a 0	h5HT1Da 0	h5HT1Db 0	h5HT1E 0	h5HT1F 0	h5HT2 0	h5HT7 0

FIGURE 1

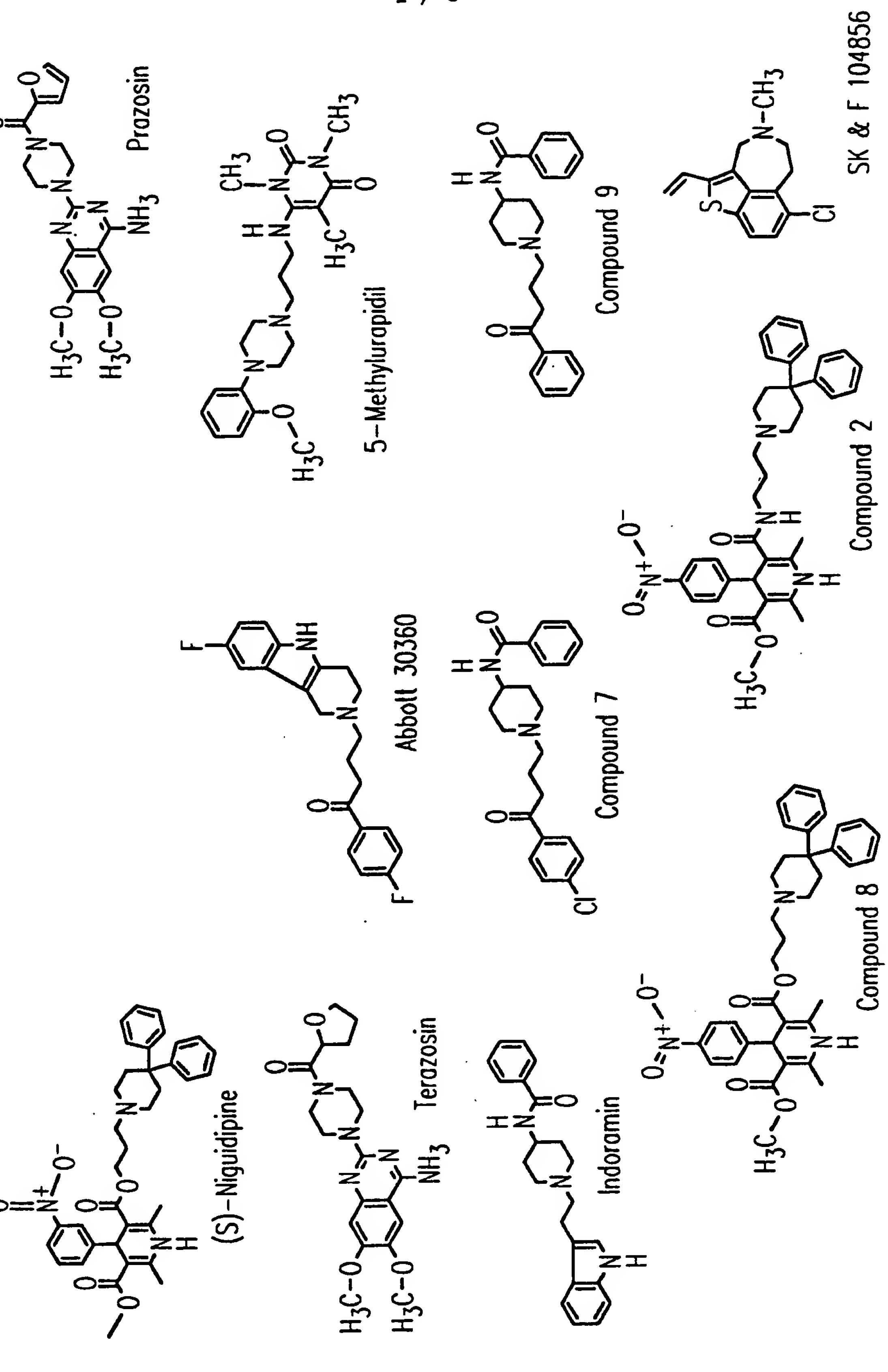

Prazosin
5-Methylurapidil
Compound 9
SK & F 104856
Abbott 30360
Compound 7
Compound 2
(S)-Niguidipine
Terazosin
Indoramin
Compound 8

FIGURE 2A

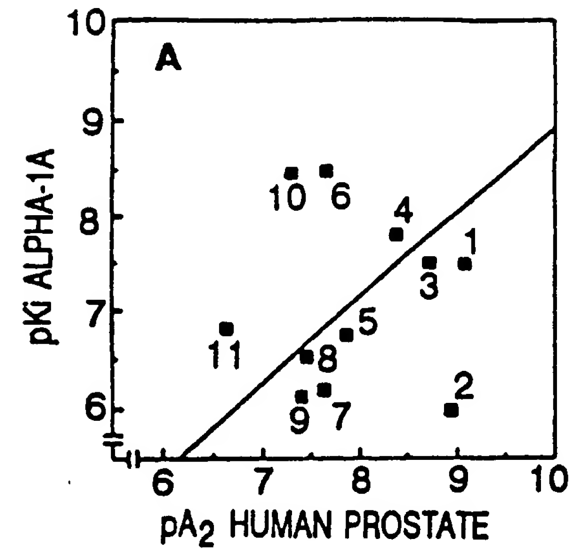


FIGURE 2B

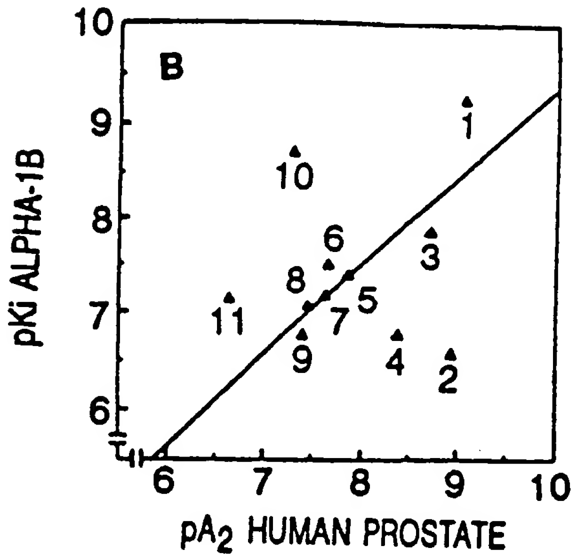


FIGURE 2C

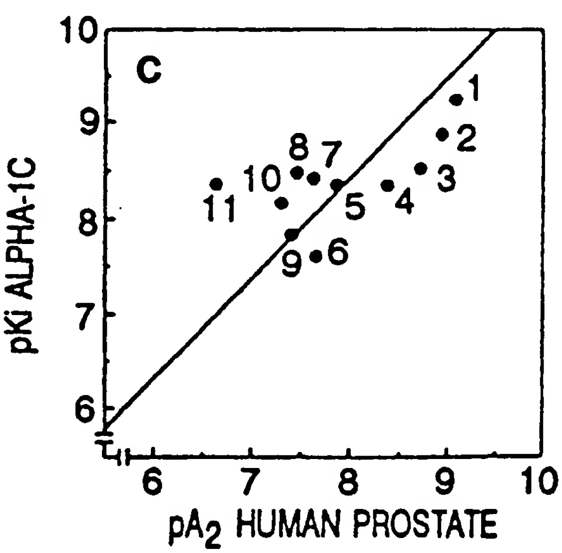
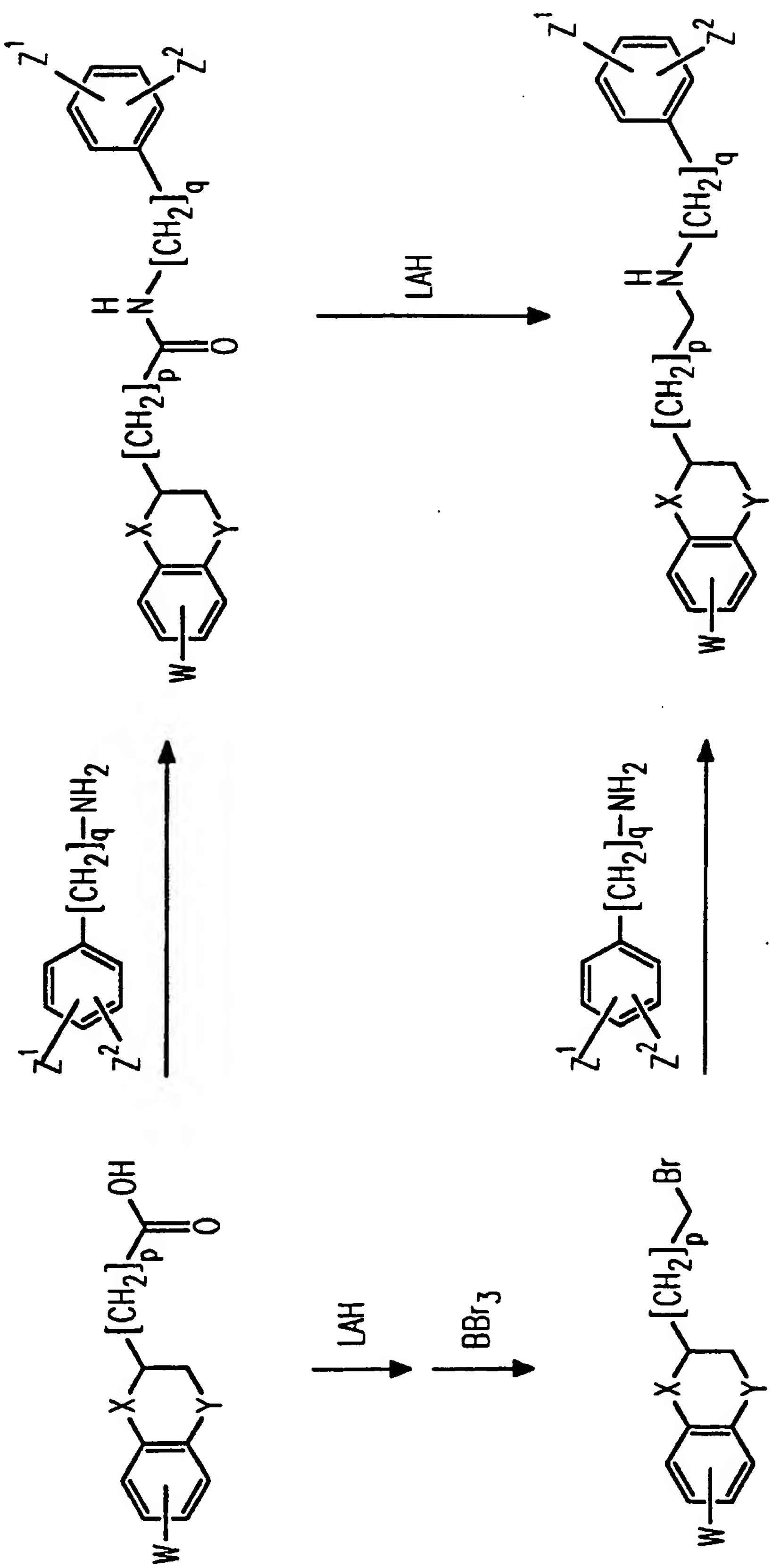


FIGURE 3



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/10162

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE, structure search.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 5,137,901 (JUNGE ET AL.) 11 August 1992, example 7.	1-18
X	US, A, 5,180,746 (GOTO ET AL.) 19 January 1993, column 1, lines 23-45.	1-18
X	US, A, 5,194,450 (MCDERMED ET AL.) 16 March 1993, column 15, line 51, compound XXXIV.	1-13
X	EP, A, 259,782 (PSIORZ ET AL.) 09 November 1986, pages 3-5.	1-13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 NOVEMBER 1994	28 NOV 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer RICHARD L. RAYMOND Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/10162

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 31/135, 31/165, 31/18, 31/195, 31/24, 31/275, 31/38, 31/385, 31/40, 31/415, 31/425, 31/47, 31/50, 31/54; C07C 211/03, 229/38, 237/30, 255/58, 311/40; C07D 209/14, 215/12, 235/14, 241/42, 277/64, 279/16, 333/58, 335/06, 339/02, 339/08.

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

514/227.5, 249, 311, 312, 367, 393, 415, 418, 432, 434, 440, 443; 544/51, 52, 353; 546/165; 548/152, 179, 309.7, 491; 549/15, 23, 32, 49, 53, 54, 58; 558/422; 560/37; 562/442; 564/86, 164, 378, 387.

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

514/227.5, 249, 311, 312, 367, 393, 415, 418, 432, 434, 440, 443; 544/51, 52, 353; 546/165; 548/152, 179, 309.7, 491; 549/15, 23, 32, 49, 53, 54, 58; 558/422; 560/37; 562/442; 564/86, 164, 378, 387.